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Vol. 5 January-February 1961 No. 1

Diagnostic Value of Cells of Endometrial and Ovarian Origin in Human Tissue Cultures. *

GEORGE N. PAPANICOLAOU, M.D., PH.D., FRANCES V. MADDI, B.A.

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THE OBSERVATIONS reported here were made during a six-year study on the behavior and the potentialities for differentiation and growth of normal, benign and malignant endometrial cells in tissue cultures. Material was obtained by aspiration and during curettage or hysterectomy. The findings in these examinations and a detailed description of the technics used were published in previous papers.[3,4] The salient points may be summarized as follows:

1. Growth activity was observed in both epithelial and stromal cells during all phases of the normal menstrual cycle.

2. Both types of cells exhibited phagocytic action and showed a tendency to adhere to and follow the pathways of cotton or other fibers experimentally introduced into the cultures. However, each of the two cell types behaved differently: the stromal swarmed around the fibers in large numbers and assumed a pattern suggesting group phagocytosis, as if trying to dispose of these foreign elements, while the epithelial cells glided around the fibers in a single layer and in a regular fashion just as the normal epithelial cells do as they spread over the endometrium's naked surface in order to form a new epithelial lining at the end of each menstrual phase (Fig. 1, 2). It is thus evident that the differentiation and growth of the endometrial cells *in vitro* are governed largely by intrinsic factors which differ in each of the two cells types.

3. Ciliated epithelial cells were observed in a fair number of living cultures with the ciliary activity continuing for a period of three to ten days. Their presence was noted chiefly in cases of polypoid hyperplasia or benign endometrial polyps.

4. Explants from benign polyps or malignant neoplasms of the endometrium displayed very active growth *in vitro*. In the normal, the highest rate of growth was observed during the proliferative phase of the menstrual cycle.

5. In contrast to the normal cells or to those of benign polyps, the cells growing out of malignant explants exhibited

* This work has been aided by a grant from the American Cancer Society, Inc., New York, N. Y.

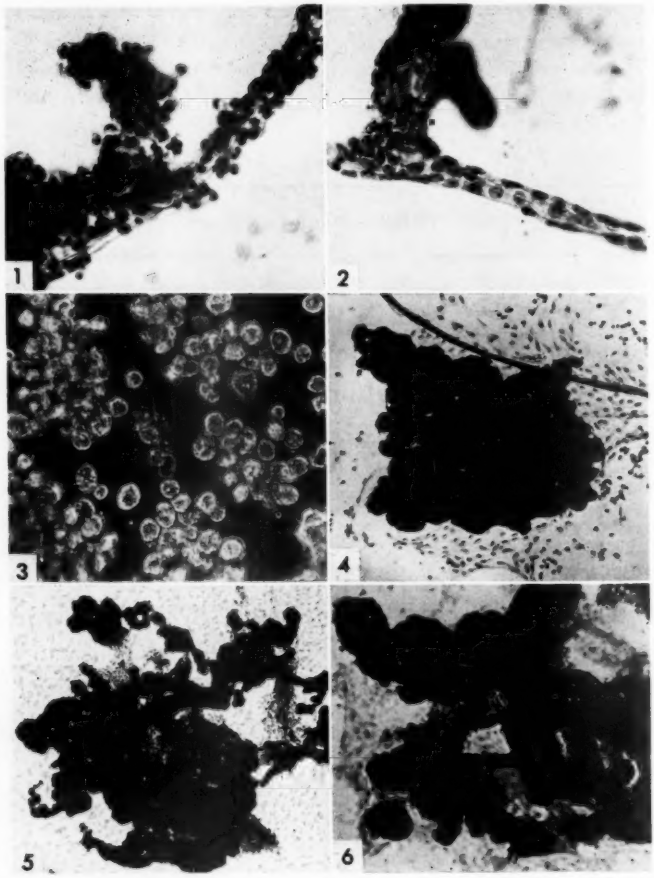

FIG. 1. 3-day endometrial tissue culture demonstrating swarming of stromal cells around cotton fibers (x240). FIG. 2. 4-day endometrial tissue culture demonstrating migration of epithelial cells around cotton fibers (x 240). FIG. 3. 8-day living tissue culture of an adenocarcinoma of the endometrium demonstrating direct growth pattern with detached cell clusters forming new centers of growth (x240). FIG. 4. 48-hour endometrial tissue culture demonstrating direct epithelial growth pattern of a normal explant corresponding to the seventh day of the menstrual cycle (x60). FIG. 5. 5-day tissue culture of a benign endometrial polyp showing direct epithelial growth pattern (x60). FIG. 6. 3-day tissue culture of an adenocarcinoma of the endometrium exhibiting direct epithelial growth pattern (x60).

marked structural abnormalities, such as a notable variation in the size of the cells and of their nuclei, a richer chromatin content and an increased mitotic and phagocytic activity.

6. The identification of malignant cells appears to be somewhat easier and more dependable in tissue cultures than in cytologic smears because of the absence of degenerated and distorted cell forms, which often cause false-positive evaluations in smears. Since cellular growth may appear in a tissue culture within 16 to 48 hours, the study of the behavior of the cells *in vitro* may be very helpful in cases in which no definite diagnosis can be established through the use of other methods.

The usefulness of the tissue culture method in the diagnosis of cancer has been further investigated in two groups of patients: one, consisting of 50 cases with pathologically proven adenocarcinoma of the endometrium, and another, of 25 cases of peritoneal and pleural effusions or washings from proven adenocarcinoma of the ovary.

Material

The material used in this investigation was obtained largely from the Lying-In Hospital of Cornell Medical Center and, to a lesser extent, from Bellevue Hospital, French Hospital and Memorial Hospital. It consisted of human endometrial tissue obtained by aspiration, curettage or hysterectomy and of peritoneal fluid and washings aspirated during operations.†

The technics used for processing this material and preparing the tissue cultures have been somewhat different from those used in the earlier studies. A description of these was given in our first publication. The most important departure has been the additional use of the roller tube technic, which proved to be particularly suitable in the application of this method in cancer diagnosis.

The general procedures followed in this study were as follows: the endometrial aspiration, which consisted for the most part of tissue fragments and loose cells, was placed in a small sterile jar containing tyrode solution. The cells may be kept in this solution for a number of hours, if necessary. Sodium penicillin and streptomycin calcium chloride were added to give a final concentration of 250 units of each per cubic centimeter, though many of our experiments have been carried out without the use of antibiotics. The inherent sterile condition of the endometrium adds to the practicability of this technic.

The loose cells and tissue fragments are implanted on 22 x 11 rectangular squares in a medium consisting of two drops of: one part fowl plasma, one part 50 per cent embryo extract and three parts human placental cord serum. The culture is allowed to clot and adhere to the coverslip before one cc. of a 50 per cent embryo extract and three parts of human cord serum are added. Within 16 to 24 hours there is, as a rule, growth of endometrial cells. The rectangular square is then removed aseptically and placed in a staining dish containing ether-alcohol fixative. Most of our cultures were stained with hematoxylin and counterstained with Orange-G. This whole procedure requires approximately 45 minutes. If the endometrial aspiration is very cellular another coverslip may be inserted into the same roller tube. Additional cultures for continued periodic examinations may thus be obtained.

In a simple endometrial aspiration, when properly obtained, as many as eight to ten roller tubes may be set up and about six Maximow cultures. If the material is abundant, it is advisable to prepare as many cultures as possible, particularly in the doubtful cases. Peritoneal effusions and washings have also been cultured in roller tubes using the same technic. When the fluid is very cellular, the specimen, instead of being centrifugated, may be left standing for awhile. One cc. of the sediment, which collects at the bottom of the receptacle, plus one cc. of the effusion fluid as a culture medium has rendered excellent results. In cultures prepared in this way, growth has been evident within 16 to 24 hours. In the preparation of Maximow cultures, centrifugation of the fluid is advisable. With regard to pleural or peritoneal washings, a mixture of half the washing, if cellular, and half of the regular medium used for the endometrial cultures has also proved successful. Cultures from effusions may retain their viability for as

† The authors wish to express their gratitude to Dr. R. Gordon Douglas and his Staff for their kind cooperation in making available to us material from patients of the Lying-In Hospital and to Dr. Elmer Kramer for placing at our disposal pathology sections with which we were able to correlate our findings. We also wish to express our appreciation to Dr. Mortimer Speiser and Dr. Cyril Solomon of French Hospital and to Dr. Gordon W. Douglas, Director of the Gynecology Division of the Bellevue Hospital. In addition, we wish to thank Mr. Constantine Railey for his valuable assistance in the selection and preparation of the photomicrographs as well as to Miss Joan Farrell for her assistance in the preparation of this manuscript.

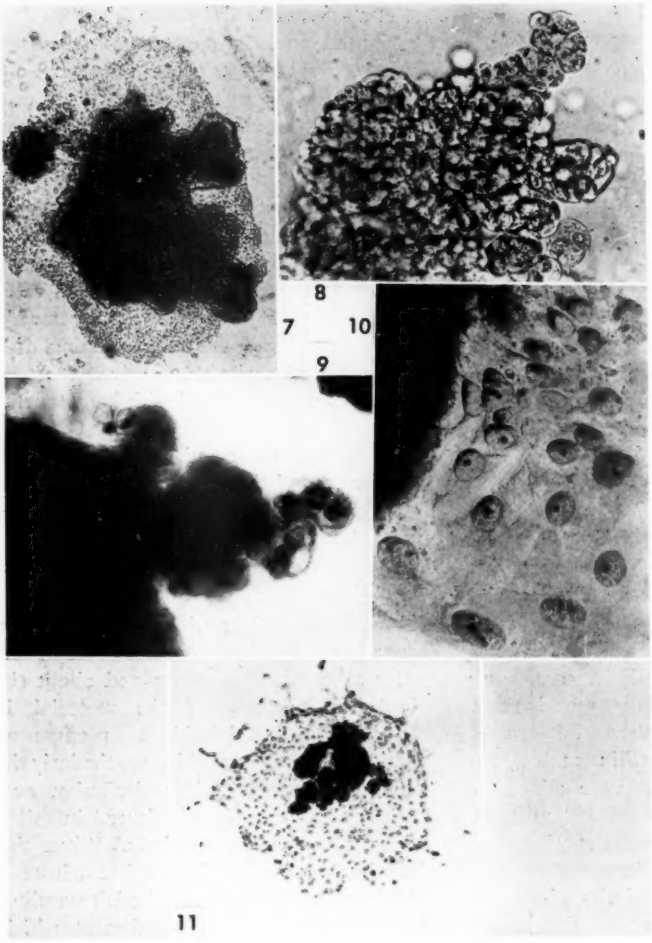

FIG. 7. 7-day living endometrial tissue culture of a case diagnosed pathologically as an early adenocarcinoma of the endometrium demonstrating a direct growth pattern in the form of rounded protrusions and an outgrowth forming a sheath of flattened cells (x60). Specimen obtained by endometrial aspiration. FIG. 8. Another 7-day tissue culture from the same case as above showing direct growth pattern (x 230). FIG. 9. 4-day tissue culture of an adenocarcinoma of the endometrium showing a direct growth pattern (x460). FIG. 10. Same culture as above demonstrating outgrowth of cells from the same malignant explant (x460). FIG. 11. 63-hour tissue culture of an adenocarcinoma of the endometrium illustrating an aggregate of cells emanating from a central core of densely packed cells (x60).

long as 30 days, without change of medium.

Liquefaction of the medium is not an uncommon occurrence in tissue cultures of the endometrium. Inactivation of the human cord serum has helped to eliminate this reaction to a certain degree. Another type of medium,* which has been tried experimentally, appears to be rather promising since it permits the tissue fragments to be kept intact after washing, thereby preserving undisturbed the structural pattern of the cellular outgrowth of the explant.

Comparative Value of the Maximow and Roller Tube Technics

Although extensive use of the roller tube technic was made in this study, it was found that much could be gained by a

* One part of a mixture of 50 per cent chick embryo extract and 50 per cent beef amniotic extract and three parts of a mixture of 50 per cent human placental (cord) serum and 50 per cent of basal medium (Eagle).

parallel use of the Maximow technic. Each has, apparently, certain advantages over the other. In the roller tube technic, there is less detail involved in the maintenance and preservation of the cells. Furthermore, little or no change of the medium is necessary and definite results are obtainable within a relatively short period of time. Therefore, many more specimens may be available for study. Another significant point is that the cultures may be left undisturbed for a longer period of time, as many as ten days, without any appreciable deterioration. However, since a number of cell clusters or aggregates adhere to the rounded wall of the roller tube, their ex-

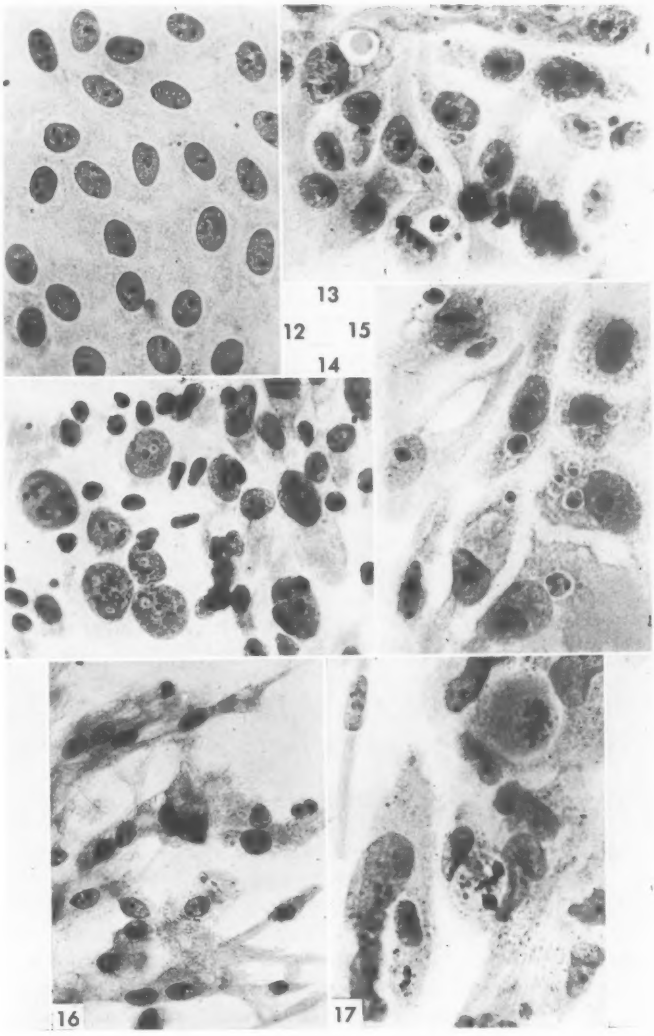

FIG. 12. Normal epithelial sheath cells from a 4-day endometrial tissue culture corresponding to the 8th day of the menstrual cycle (x465). FIG. 13. Sheath cells from a 7-day tissue culture of an adenocarcinoma of the endometrium (x465). Note large nucleoli. FIG. 14. Sheath cells from a 5-day tissue culture of a papillary adenocarcinoma of the endometrium (x465). Note markedly abnormal nuclei FIG. 15. Sheath cells from a 5-day tissue culture of an adenocarcinoma of the endometrium (x465). Note phagocytosis of degenerating inflammatory cells and prominent nucleoli. FIG. 16. Normal stromal cells from a 48-hour endometrial tissue culture (x465). Compare with Fig. 2, which shows normal epithelial cells. FIG. 17. Sheath cells from a 12-day tissue culture of a carcinosarcoma of the endometrium (x465). Note nuclear abnormalities, mitosis and phagocytic activity. Specimen obtained by endometrial aspiration.

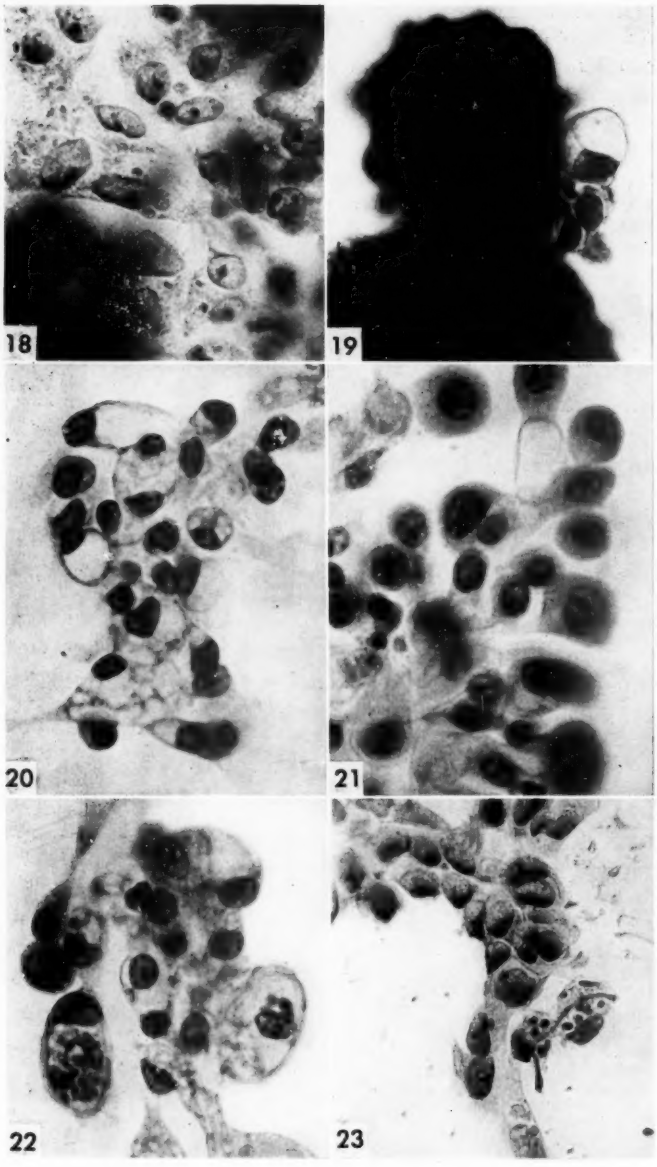

FIG. 18. Sheath cells from a 7-day tissue culture of an adenocarcinoma of the endometrium. Same case as in Fig. 13 (x540). Note large nucleoli and polarization of the nuclear chromatin. FIG. 19. 6-day tissue culture of an adenocarcinoma of the endometrium (x540). Note vacuolated cells breaking away from a growing explant. FIG. 20. 3-day tissue culture of an adenocarcinoma of the endometrium showing generalized vacuolation (x540). FIG. 21. 20-hour roller tube tissue culture of peritoneal washings from a case of a papillary serous cystadenocarcinoma of the ovary (x540). Note squamous metaplasia as seen in cells of the adenoacanthoma type. FIG. 22. 16-hour roller tube tissue culture of an adenocarcinoma of the endometrium. Specimen obtained by endometrial aspiration (x540). Note vacuolation and phagocytosis of detritus. FIG. 23. 48-hour jar tissue culture of an adenocarcinoma of the endometrium (x540). Note phagocytosis of monilia spores and a mycelium by adenocarcinoma cells.

amination or photography under higher magnifications becomes difficult.

The Maximow culture, although in a way more involved in its preparation and maintenance, permits a more comprehensive study of both the living explant and the fixed and stained specimen. One can thus better evaluate the general con-

figuration and structural pattern of the explant and its growth potential. On the other hand, the roller tube culture lends itself better to use as a routine diagnostic test. A combination of the two, whenever possible, is, therefore, very desirable.

Cytology of Tissue Cultures in Adenocarcinomas of the Endometrium

A general description of the normal and malignant cytology of the endometrial mucosa, as seen in fixed and stained tissue cultures, can be found in our previous papers. The description given here, though supplementary, is, however, more specific as it covers in greater detail the diagnostic aspects and potentialities of the tissue culture technic.

In examining a culture of malignant endometrial cells, one is always impressed with their high growth activity. In the undisturbed living culture, there is, as a rule, continual outgrowth of cells, many of which become detached from the surface of the explant either singly or in the form of sheaths, aggregates or clusters of cells. Such cell groups as they move away from the explant may become new centers of expansion and growth (Fig. 3). A more typical picture of this can be seen in the Maximow culture. The growth patterns are essentially the same in both types of cultures with a relatively limited variability. During the process of fixation and staining, there is always a notable loss of free cells and clusters often conveying the impression that the growth activity is much less pronounced in the stained as compared with the living culture. This is especially noticeable in the Maximow cultures.

As previously explained,[3, 4] it is possible, in endometrial tissue cultures, to distinguish two different growth patterns: one, representing a direct growth of the peripheral cells of the explant, in the form of rounded or spherical protrusions or of digital projections; and another, consisting in an outgrowth of cells rushing out of the explant in the form of a sheath of flattened cells, which, as they spread out, tend to grow considerably larger (Fig. 7-10). A similar outgrowth pattern can also be seen in roller tube cultures. In such cultures, the cells appear mostly in the form of aggregates, some of which may have a central core of densely packed cells (Fig. 11). The growth activity often appears to be more pronounced in the roller tube cultures probably because of the prevalence of a more favorable nutritional environment.

Although the structural details of the cells are more clearly outlined in the fixed and stained cultures, several distinctive traits and patterns of the malignant cells may also be discerned in their living state. These can be seen chiefly in cultures which have not been contaminated with polymorphonuclears and other inflammatory cells. Many of the adenocarcinoma cells observed *in vitro* exhibit distinctly abnormal features, which reveal their malignant nature, while others tend to display very atypical yet not conclusively malignant forms. In some of the malignant cases, particularly in their early stages, a granular material was sometimes noted within the cells of the explant (Fig. 8). One should also always expect to find in a tissue culture preparation a number of normal appearing cells, which, by virtue of their contrast, may, in a way, facilitate the detection and identification of the malignant cells.

The above described changes, as seen in the living cells, may often be sufficiently characteristic to permit the identification of their malignant nature. However, the cytologic as well as the histopathologic criteria of malignancy are usually more clearly defined in fixed and stained cultures and much more consistent with those generally accepted in any cytology or pathology laboratory. Of these criteria, the most important are those related to the structure of the nucleus. Karyomegaly, true hyper-

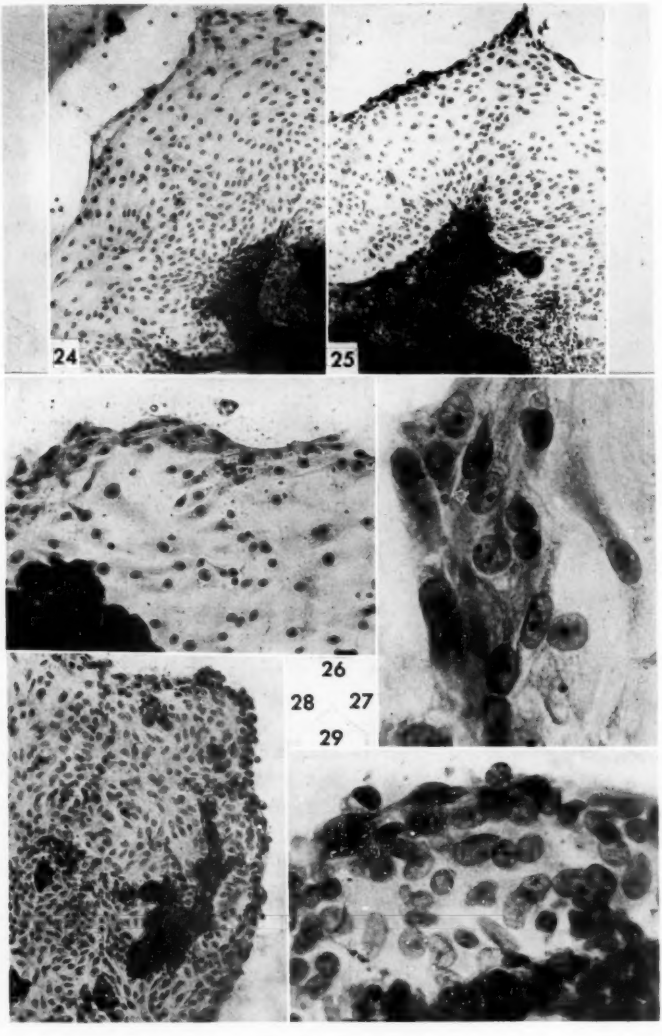

FIG. 24. 4-day normal endometrial tissue culture corresponding to the 8th day of the menstrual cycle (x75). Note extrusion of epithelial cells from gland openings. Cells from the same case at x465 magnification are illustrated in Fig. 12. FIG. 25. 5-day tissue culture of a papillary adenocarcinoma of the endometrium (x75). Note extrusion of malignant cells and a superficial zone of dense elongated cells. FIG. 26-27. 3-day tissue culture of an adenocarcinoma of the endometrium (Fig. 26, x115. Fig. 27, x465). Note formation of dense superficial zone. FIG. 28-29. 5-day roller tube tissue culture of an adenocarcinoma of the endomentrium (Fig. 28, x 115. Fig. 29, x465). Note formation of dense superficial zone. Specimen obtained by endometrial aspiration.

chromasia due to excess of the chromatin content, abnormal nuclear forms, multinucleation, mitotic activity with aberrant types, amitotic divisions or nuclear budding, atypical distribution or polarization of the chromatin and unusually large nucleoli are all criteria which have as much significance in this as in any other microscopic examination for cancer diagnosis. Some of the nuclear abnormalities most frequently observed in tissue culture preparations can be seen in Figures 13-15, 17-18. In adenocarcinomas of the endometrium, the presence of a single large nucleolus, as shown in Figures 13 and 15, is probably as valid as any other single criterion of

malignancy. The above enumerated nuclear abnormalities of malignant cells are common to both adenocarcinomas and sarcomas of the endometrium.

The changes observed in the cytoplasm of the malignant cells in fixed and stained cultures, though not as pathognomonic as those seen in the nucleus, are quite often of definite diagnostic value. One of these changes is the atypical and often pronounced vacuolation of the malignant endometrial cells. In tissue fragments or cell clusters, the vacuolation may be confined to the superficially located cells (Fig. 19) or may affect every cell of the explant (Fig. 20). An irregular architectural pattern and a loss of the boundaries between the cells are also among the criteria observed in sheaths, aggregates or clusters of malignant cells. In cases of adenoacanthoma, larger metaplastic cells with malignant features may be found in tissue cultures in addition to the adenocarcinoma cells.

The phenomenon of vacuolation appears

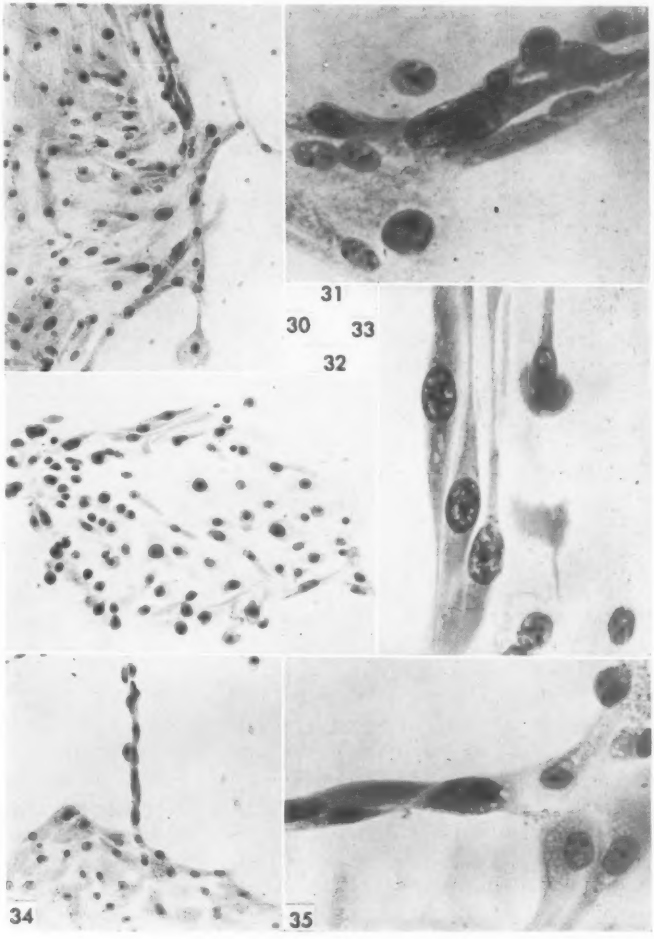

FIG. 30-31. 5-day tissue cultures of adenocarcinoma of the endometrium (Fig. 30, x115. Fig. 31, x 465). Note dense peripheral zone. FIG. 32-33. 42-hour roller tube tissue culture of peritoneal washings from a case of a papillary cystadenocarcinoma of the ovary (Fig. 32, x115. Fig. 33, x465). Same case as Fig. 21. FIG. 34-35. 9-day tissue culture of an adenocarcinoma of the endometrium demonstrating the streaming out of cells from the dense zone. (Fig. 34, x115. Fig. 35, x465).

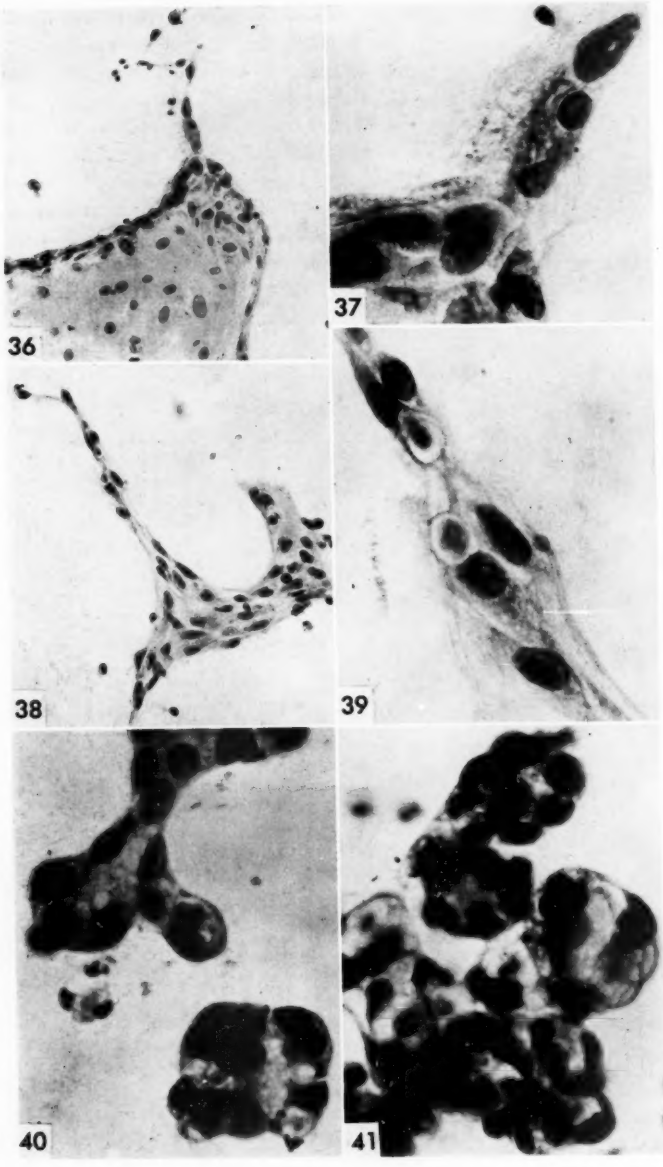

FIG. 36-37. 4-day tissue culture of an adenocarcinoma of the endometrium showing dense zone with outgrowing cells (Fig. 36, x135. Fig. 37, x540). FIG. 38-39. 72-hour tissue culture of an adenocarcinoma of the endometrium illustrating the same phenomenon as above (Fig. 38, x135. Fig. 39, x540). Specimen obtained by endometrial aspiration. FIG. 40 72-hour roller tube culture of ascitic fluid from a primary serous cystadenocarcinoma of the ovary with metastases to the omentum (x540). FIG. 41. 48-hour roller tube culture from the same case as Fig. 40 (x540).

to be closely associated with a high phagocytic activity, which is one of the characteristics of the malignant endometrial cells. The adenocarcinoma cells are rather indiscriminate in their phagocytic action and can engulf and digest other smaller cells, particularly degenerating blood cells, cellular detritus and various parasitic and

bacterial forms (Fig. 22, 23). This unusually high phagocytic potentiality of the malignant endometrial cells may be considered as an accentuated expression of a fundamental biological property inherent in the normal epithelial cells of the endometrium, that of differentiating into true phagocytes. Cells liberated from the epithelium during the postmenstrual and early proliferative stages may thus assume the important function of cleansing the uterine cavity of the detritus accumulating after each menstrual exfoliative process.[5] One should, of course, be sure not to interpret as malignant the smaller phagocytic cells which are rather frequently seen in tissue cultures, both malignant and nonmalignant. These cells have a normal appear-

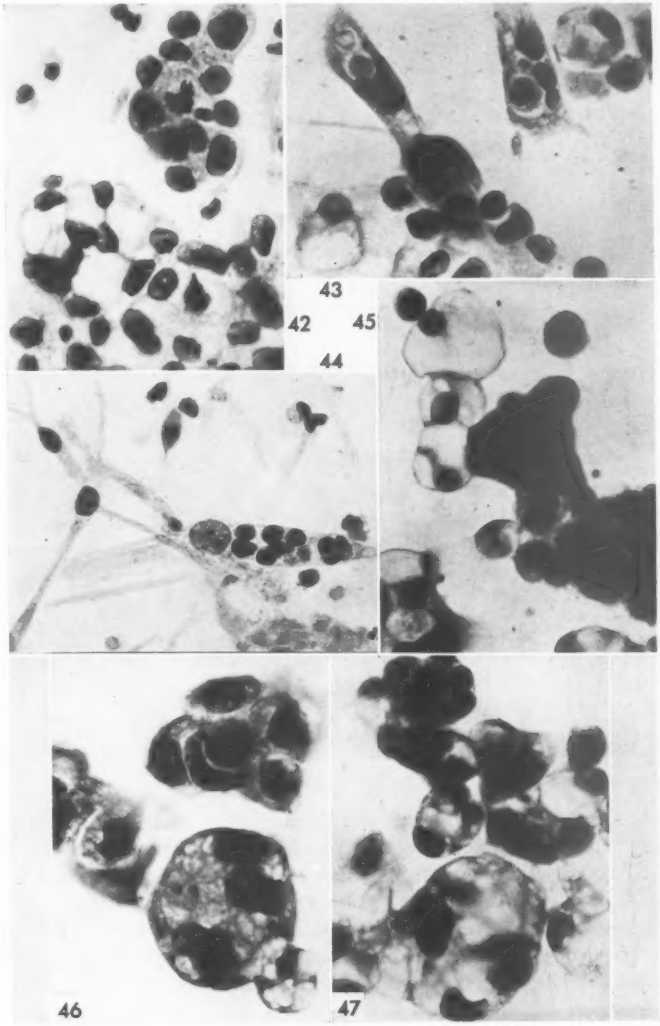

FIG. 42. 16-hour roller tube culture from the same case as Fig. 41 (x 465). Note the mitotic figure. FIG. 43. 4-day tissue culture of adenocarcinoma of the endometrium (x465). Note phagocytosis of smaller cells possibly mononuclears. Specimen obtained by endometrial aspiration. FIG. 44. 3-day roller tube tissue culture of ascitic fluid from a case of generalized carcinomatosis (x465). Note phagocytosis of inflammatory cells, also two normal fibroblasts. FIG. 45. 48-hour roller tube culture of ascitic fluid from the same case illustrated in Fig. 40-42 about one month following irradiation (x465). Note extreme vacuolation of three cells growing out of a cluster consisting of well preserved and apparently viable cells. FIG. 46. 5-day roller tube tissue culture of ascitic fluid from a case of metastatic papillary serous cystadenocarcinoma of the ovary showing very pronounced vacuolation and degeneration of cells approximately 4 months following irradiation (x 465). FIG. 47. Same case and culture as Fig. 45. Note marked radiation effect on some of the cells (x465).

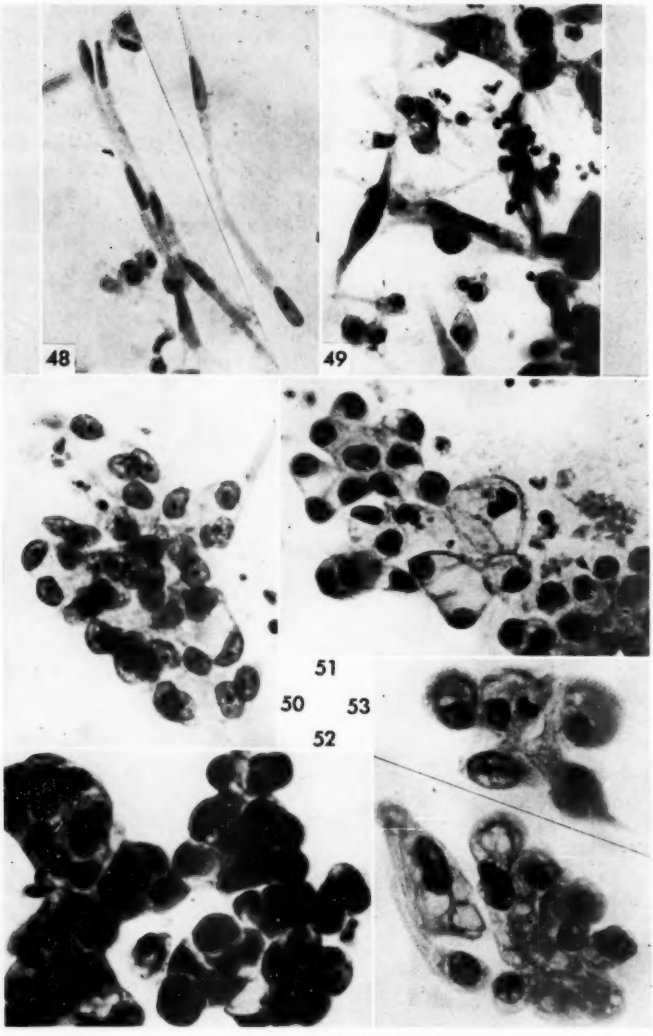

FIG. 48. 42-hour tissue culture from a specimen of a mixed mesodermal tumor obtained during operation (x465). The narrow elongated cells are interpreted as endothelial. FIG. 49. 16-hour roller tube tissue culture of a peritoneal washing demonstrating normal cells interpreted as fibroblasts intermixed with inflammatory cells (x465). FIG. 50-51. 16-hour and 4-day roller tube tissue cultures of endometrial aspiration fluid from a case of adenocarcinoma. This is one of the cases in which the tissue culture findings have contributed to the diagnosis (x465). FIG. 52. Same case and culture as Fig. 45, 47 showing a persistence of viable malignant cells after irradiation (x465). FIG. 53. 6-day roller tube tissue culture of an irradiated adenocarcinoma of the endometrium showing viable cells after a treatment of approximately 7 days (x 465). Specimen obtained by endometrial aspiration.

ance and normally phagocytose degenerating inflammatory cells, erythrocytes as well as bacteria and parasites.

Actively growing explants from a benign polypoid or papillary growth often assume a pattern greatly resembling that of an endometrial adenocarcinoma (Fig. 4-6). However, despite the close similarities in their growth patterns, the benign and malignant cells are apparently endowed with different potentialities for differentiation, which determine their final configuration into one or the other type. The behavior of these two cell types in tissue cultures indicates that each of the two factors, the one stimulating growth and the other re-

sponsible for malignancy, though generally combined in their action, actually represent two distinct entities.

The structural details and the differential characteristics of both malignant and non-malignant cells can be more clearly outlined in the sheaths formed by the cells growing out of the tissue. These cells are, as a rule, flattened, enlarged and more transparent than the densely packed cells within the explant. In such sheaths, malignant cells, if present, can be more easily identified by their aberrant features (Fig. 13-15 and 17-18).

Sheath cells with somewhat atypical structural characteristics may occasionally be seen in non-malignant cultures, chiefly in cases of hyperplasia in which a certain variation in the size of the nuclei (anisokaryosis) is not an uncommon occurrence. The structure of the individual cells in such cases is, however, essentially normal.

What enhances the diagnostic value of the tissue culture method is that the individual characteristics of the malignant cells and their growth patterns, as seen in fixed and stained cultures, are, in many instances, very clearly defined. A conclusive and definitely positive diagnosis, equivalent to a Class V cytologic evaluation is thus possible in a fair number of cases. In addition to the above described structural abnormalities, there may also be a characteristic elongation and densification of the cells. This change is more noticeable in the peripheral cells, which then tend to spread over the surface of the explant or aggregate in the form of a narrow compact zone (Fig. 25-33). In certain areas, some of these superficial elongated cells stream out in a perpendicular fashion suggesting a migratory activity (Fig. 34-39). A variety of other abnormal features as well as an intense phagocytic activity may also be often noted in such cells (Fig. 39).

Cytology of Tissue Cultures from Pleural and Peritoneal Effusions

The abnormal features of malignant cells found in tissue cultures from aspirations of pleural or peritoneal fluid or washings in cases of metastatic adenocarcinoma show great resemblance to those described for the adenocarcinomas of the endometrium (Fig. 13-15 and 17-18). The cytologic criteria by which the presence of a malignant process can be ascertained are, therefore, essentially the same.

In general, both the proliferative and growth activity of the malignant cells are very marked in tissue cultures from pleural and peritoneal fluid or washing specimens. Mitotic and amitotic division or budding are not uncommon in such cultures. One can also find a variety of aberrant and bizarre cell forms and a high phagocytic activity (Fig. 40-44). Since pleural or peritoneal fluid is in itself an excellent culture medium, it may be used as such with very good results either alone or in combination with equal parts of the regular medium. Extreme vacuolation, as already mentioned, is known to be one of the most characteristic postradiation changes (Fig. 45-47).

Elongated malignant cells, when single, can oftentimes be identified with great difficulty since effusion tissue cultures, often contain large numbers of fibroblasts and endothelial cells. The endothelial cells can be more easily recognized by their slender form and their tendency to arrange themselves in a linear formation (Fig. 48), whereas the fibroblasts present a more difficult diagnostic problem due to their great variability in size and form (Fig. 49). When grouped in aggregates or clusters, the malignant cells can be identified with much higher accuracy, equalling that of the malignant endometrial cells.

Discussion

The value of tissue culture in the diagnosis of malignant disease was advocated by a number of investigators, who used this method in the study of pleural and peritoneal effusions. Coman[2] states that "cells from carcinomas and sarcomas grew vigorously, thereby indicating that such cells remain viable and capable of proliferation after floating in pleural or peritoneal fluids if given a satisfactory surface to which they may become attached." Coman used the roller tube technic. He concludes that "the culture of cells from pleural and ascitic fluids can in some instances at least, be of diagnostic aid." Similar conclusions were reached by Sano and Bello[6] who found that "cells from human body fluids have different growth patterns in tissue culture which may be of diagnostic value, especially in those cases in which other methods have failed to establish a diagnosis." In a later report on his observations in a study of a new series of pleural effusions in correlation with "cell block and Papanicolaou smear" studies, Sano[7] states that "Kinetic pathology, or tissue culture as applied to pathology has been shown to be a valuable diagnostic tool and an adjunct to cell block and smear studies, but not a substitute for these."

Bencze[1] summarizes as follows his investigations on the use of tissue culture in the diagnosis of cancer in exudates, made with the aid of a special technic: "Diagnostic tissue cultures of thoracic and abdominal exudates were carried out in 30 cases. The tissue culture diagnosis was in agreement with the clinical diagnosis in 23 of the cases; in 6 cases the correct diagnosis was given only by the tissue culture. In one instance the autopsy diagnosis differed from that of the tissue culture. In the tissue culture, the tumor cells form large cell groups which are of value diagnostically." (Translation from German.)

The results obtained by these investigators are in agreement with our observations. The tissue culture is indeed of definite value in the diagnosis of neoplasms developing within the pleural or peritoneal cavities, more specifically of those metastasizing from primary carcinomas of the ovary. In the light of our investigations, the same statement can also be made with regard to the utilization of the tissue culture method in the diagnosis of adenocarcinoma of the endometrium. Its application in this organ is bound to be of immense value not only in establishing the diagnosis of intra-uterine cancer in some of the obscure cases but also in its early detection which is still difficult with the currently used diagnostic procedures.

Exfoliative Cytology, which proved to be of major importance in the diagnosis and early detection of cancer of the uterine cervix, has unfortunately, not been equally satisfactory in the detection of intra-uterine malignancies. Routine vaginal and cervical and even endometrial smears often fail to provide sufficiently conclusive evidence in cases in which the presence of an intra-uterine neoplasm is suspected. In these cases, the addition of a tissue culture to the routine cytologic examination would probably be of great help in clarifying the diagnosis. The two methods actually complement one another. The smear technic possesses the advantage of general applicability, which makes it an ideal routine screening and diagnostic procedure. On the other hand, a tissue culture preparation may, in some instances, add more conclusive evidence permitting a definitely positive Class V evaluation. This is more important in early malignancy in which the diagnosis is always more difficult due to the lack of clean-cut and strictly objective cytologic and histopathologic criteria for identifying the earliest manifestations of endometrial adenocarcinoma. In several of the early or ambiguous cases

of adenocarcinoma which have been included in this study, the tissue culture has provided the primary or the most conclusive evidence. In two instances a surgical procedure was decided upon largely on the basis of the tissue culture findings.

The evaluation of the effect of irradiation is another useful and very promising application of the tissue culture method. Exfoliative cytology has already been applied on a relatively large scale in the study of postradiation changes and in many instances has provided significant information regarding the effectiveness of radiation therapy and the evidence of a residual extension or recurrence. However, the usefulness of exfoliative cytology in evaluating structural changes in irradiated cells is limited by the fact that, in most instances, it is difficult to decide as to the viability of these cells and, therefore, as to the persistence of an active growth. Such information, may, of course, be provided by the examination of cells *in vitro* (Fig. 52, 53).

Adequate growth for a diagnostic evaluation of a tissue culture can often be obtained within 16 to 24 hours (Fig. 21, 22, 42, 50), although older cultures exhibiting more advanced stages of growth are, in general, more conclusive, particularly in the doubtful cases. Occasionally, the malignant cells appear for the first time in cultures several days old. Negative findings should, therefore, never be interpreted as definitely conclusive.

Endometrial cells, both normal and malignant, were found to be viable in cultures from aspiration specimens, which have been kept in refrigeration for 24 to 48 hours and, in cases of ascites for three to four days after tapping. In a number of effusion cultures continued growth was noted for a period of 25 to 30 days without change of the medium.

A rather important point with regard to the use of tissue culture as a routine diagnostic procedure, is that a simple aspirate of endometrial fluid is, in many instances, sufficient to secure adequate cytologic material for a tissue culture study. Curettage material, of course, is apt to be more copious but implies an operative procedure, which is not always advisable or feasible.

Although fully realizing the various difficulties, technical and others, associated with the wider use of tissue culture in the diagnosis of cancer, polypoid hyperplasia and other pathologic conditions of the endometrium, we feel that the continued advancement in the scientific and technical aspects of this method and its proven diagnostic reliability make it not only very desirable but well-nigh indispensable as an adjunct to other diagnostic procedures. The fact that, in several instances, a definitive diagnosis may depend largely on the utilization of this method,, fully justifies its incorporation in the armamentarium of the well equipped diagnostic pathology or cytology laboratory—not to mention its immense value in the investigation of problems not only of clinical but also of fundamental significance and import.

Conclusions

1. The value of the tissue culture method in cancer diagnosis has been investigated in 50 cases of pathologically proven adenocarcinoma of the endometrium and 25 of peritoneal and pleural effusions or washings from proven adenocarcinoma of the ovary.

2. The material consisted of human endometrial tissue obtained by aspiration, curettage or hysterectomy and of peritoneal fluid or washings aspirated during surgery.

3. Two technics were used for the preparation of cultures, the Maximow and the roller tube. The Maximow culture proved to be of greater value in the over-all study of the malignant endometrial cytology and of the prevailing growth patterns. The main advantage of the roller tube technic

has been its greater adaptability as a diagnostic procedure.

4. Observations on the morphologic characteristics and growth activity of the cells were made in living as well as in fixed and stained preparations. Malignant cells have, as a rule, exhibited very active growth and an intense phagocytic activity.

5. The cytologic criteria of malignancy were found to be comparable to those generally accepted in the pathology and cytology laboratories. However, the high growth activity of the malignant cells *in vitro* results in the appearance of a variety of characteristic patterns, which provide additional criteria for a more conclusive diagnosis.

6. The tissue culture method can be most profitably used in obscure diagnostic cases in which the presence of a malignant neoplasm could not be established by other diagnostic procedures. It also proved to be of great value in the detection of early cancer and in the evaluation of postradiation changes by ascertaining the viability of malignant cells, which have been exposed to irradiation.

7. Since growth, particularly in the roller tube cultures, may be observed within 16 to 24 hours and simple aspiration specimens can be obtained with relative ease in the majority of cases, it is felt that the tissue culture method may be employed, at least in certain organs, to great advantage as an adjunct to other diagnostic procedures.

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Clinical Cytology for Pathologists—Postgraduate Course

The Second Institute on Clinical Cytopathology is to be given at the Johns Hopkins University School of Medicine and Hospital, Baltimore, Maryland, March 20-25, 1961. The full six-day program is designed exclusively for Pathologists who are Certified (or qualified) by the American Board of Pathology (PA). All aspects of clinical cytopathology will be covered.

The first three days will be an intensive refresher of the field. The last three days will be devoted to special problems, newer technics and recent applications; these topics will be explored in small informal conferences over the microscope with the Faculty. A loan set of slides with text, will be sent to each participant weeks before the Institute, for home study.

Application is to be made before February 15, 1961; acceptance will be on a "first come, first served" basis. For details, write: John K. Frost, M.D., 610 Pathology Building, Johns Hopkins Hospital, Baltimore 5, Maryland.

Dysplasia in the Uterine Cervix During Pregnancy: An Analytical Study of the Cells

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The dysplastic reaction which occurs in the uterine cervix of the pregnant woman is of considerable biological importance. It may be detected prior to or possibly antedate the development or actual demonstration of invasive or *in situ* cancer. It may persist for varying periods of time without undergoing significant change or may persist and become progressively more severe. It may undergo regression or at least is not apparent after pregnancy is terminated. Because of the variability of its biological behavior and its occurrence in relatively young patients it may be an early indication of the stimulus which under certain still unknown conditions may bring about cancer.

In an attempt to gain more knowledge about the cellular changes observed in the presence of dysplasia this detailed analytical study was undertaken. The purpose of the study is: (1) to determine the occurrence of the reaction in a pregnant population, (2) to analyze the cellular changes in detail, (3) to correlate the cellular and histopathological alterations, and (4) to study the cellular sample in relation to the ultimate outcome of the lesion.

Materials and Methods

The material utilized in this study was obtained from 1,000 consecutive new admissions to the Maternity Out Patient Department of the University Hospitals of Cleveland. Over a period of ten months all patients were studied on their initial visit. Of the total represented only 930 had one or more subsequent examinations in the postpartum period.

Cellular samples were collected from the uterine cervix by scraping the portio vaginalis and aspirating the contents of the endocervical canal. The material was transferred to glass slides, fixed in a solution composed of equal parts of 95 per cent ethyl alcohol and ether, and stained with a polychrome technic.

Those preparations having any significant degree of abnormality were subjected to detailed examination. In an attempt to obtain a random sample, each slide was examined using a systematic-meander screening pattern designed to cover the entire slide. Cells were analyzed at regularly spaced intervals along the screening pattern until a minimum of 100 cells were studied. Measurements of the greatest nuclear and cellular diameters were made by using a calibrated ocular micrometer. Areas were computed on the basis of these measurements.

All patients having significant cellular abnormalities were investigated by multiple cell studies and four quadrant punch biopsies. The patients having significant abnormalities were followed until the fate of the lesion was known or a minimum of five years had elapsed.

Results

Population Studied: Of the 930 patients represented in this series, 834 or 89.7 per

cent were Negroes and only 96 or 10.3 per cent were white. At time of initial study 181 or 19.4 per cent were in the second decade of life, 562 or 60.4 per cent were in the third decade, 170 or 18.2 per cent were in the fourth decade, and 17 or 1.8 per cent were in the fifth decade. When first examined, 325 or 34.9 per cent were in the first trimester of pregnancy, 558 or 60.0 per cent were in the second trimester, and 47 or 5.1 per cent were in the third trimester. A total of 316 or 34.0 per cent of the patients were para 0, 230 or 24.7 per cent were para 1, 183 or 19.7 per cent were para 2, 98 or 11.0 per cent were para 3, and 103 or 10.5 per cent were para 4 or greater.

Occurrence

Some degree of cellular abnormality was observed in preparations from 58 or 6.2 per cent of the 930 women examined. Abnormalities were seen in 53 or 6.3 per cent of the Negroes and in 5 or 5.3 per cent of the white women. The cellular changes were interpreted to be of minor significance in 30 of the 58 women. In 28 or 3 per cent of the patients studied, cellular changes were interpreted as being indicative of a dysplastic reaction. This was subsequently confirmed by so-called "punch" biopsy. Of the lesions demonstrated 25 were in Negroes, accounting for 3 per cent of those surveyed and three were in white women with a frequency of 3 per cent. An occurrence rate of 3.1 per cent were observed in a comparable population of 10, 836 pregnant women studied by cellular samples. This attests to the validity of the occurrence rate in the smaller series in the present study. While none of the patients in the present study had cellular evidence of *in situ* cancer at time of initial sampling, 0.2 per cent of the women in the larger series had cellular change of *in situ* cancer.

TABLE 1. *Parity of Patients in Relation to Cellular Abnormalities*

Para	Cases	Dysplasia No.	Dysplasia %	Abnormality No.	Abnormality %
0	316	3	0.9	10	3.1
1	230	3	1.3	12	5.2
2	183	8	4.3	14	7.6
3	98	5	5.1	10	10.2
4*	103	9	8.7	12	11.6
Totals	930	28	3.0	58	6.2

* Or greater.

The mean age of the patients with dysplasia was 28.2 years. This is a reflection of the age distribution of the population surveyed. A dysplastic reaction was observed in 1 or 0.6 per cent of the women in the second decade of life, in 21 or 3.7 per cent of those in the third decade, in 4 or 2.3 per cent of those in the fourth decade, and in 2 or 11 per cent of the women in the fifth decade of life at time of initial study.

Of the patients initially seen in the first trimester of pregnancy, 9 or 2.8 per cent had cellular evidence of dysplasia as did 10 or 1.8 per cent of those in the second trimester and 9 or 19.2 per cent of those in the third trimester.

As shown in Table 1, the occurrence of any cellular abnormality and the occurrence of cellular changes considered to be those of dysplasia was related to the parity of the patients. With increasing parity there was a higher incidence of dysplasia. This observation is of some importance if there is a relationship between pregnancy and the development of a dysplastic reaction. It cannot be stated with certainty that the dysplastic reactions represented in the present study developed during the pregnancy. Of interest, however, was the fact that 17 of the 28 patients had previous cellular samplings obtained from two to 12 months prior to the present pregnancy. In 15 of the 17 cases the specimens were within normal limits while in two cases there was cellular evidence of a pre-existing dysplastic reaction. Both patients were known to have had previous pregnancies although there was no evidence to indicate

that the reactions had developed in a previous pregnancy.

The Cellular Sample

A variable number of abnormal cells was represented in the preparations examined. Counts of abnormal cells did not exceed 199 per slide in specimens from 12 patients. In material from three patients counts ranged from 400 to 599 cells and in preparations from seven of the patients, abnormal cells counts were in excess of 600 per slide.

Distribution of Cells

Of the cells analyzed in the systematic-meander screening pattern, 86.4 ± 7.1 per cent were isolated while 13.6 ± 1.3 per cent were component cells of a sheet. In such a grouping, the cells were regularly arranged with relationship to one another and had distinct cell boundaries. Neither in the screening pattern nor elsewhere in the slides were there syncytial arrangements of cells.

Cell Size

As determined by measurements of greatest dimension, the abnormal cells had a mean diameter of 42.4 microns and a mean computed area of 1407.7 ± 648.19 square microns. This value was somewhat higher than the mean area of 1249.8 ± 426.3 square microns recorded in a previous study.[33] This is in part explained by the fact that the present study represents a cross section of a pregnant population while the previous was based on selected cases.

In Figure 1, the size of the abnormal cells was plotted out in relation to the size of the normal squamous cells represented in the same material. The population of abnormal cells included some forms which were comparable in size to normal squamous cells observed during pregnancy. Other abnormal cells were significantly smaller than the normal squamous forms. This suggested that more than one universe

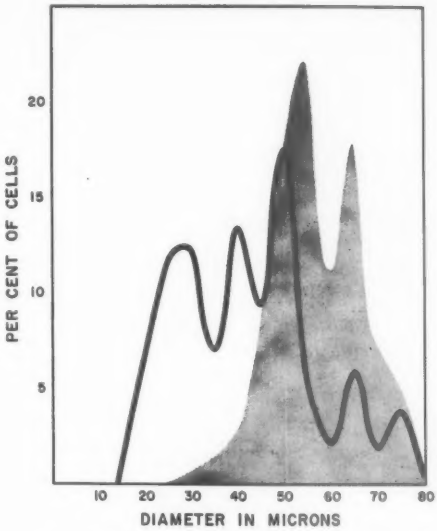

FIG. 1. Distribution of altered cells as to size (solid black line) in relation to size of normal squamous cells in pregnancy (cross hatched).

was represented in the surveyed population of abnormal cells.

On examining the distribution curves of cell measurements in the individual cases, there were five in which the abnormal cells were comparable in size to normal squamous cells. In nine cases almost all of the abnormal cells were significantly smaller than the measured squamous cells. In 14 cases there were significant numbers of large, intermediate, and small cells represented in the cellular sample.

When considered in relationship to their histopathological reaction of origin, the large abnormal cell forms as illustrated in Figure 2, having an area comparable to that observed for normal squamous cells, were more commonly related to a reaction as illustrated in Figure 3. Cells of intermediate size, as depicted in Figure 4, having a diameter of about 40 microns were more commonly observed in the presence of a reaction as illustrated in Figure 5. Such a reaction usually was observed along with

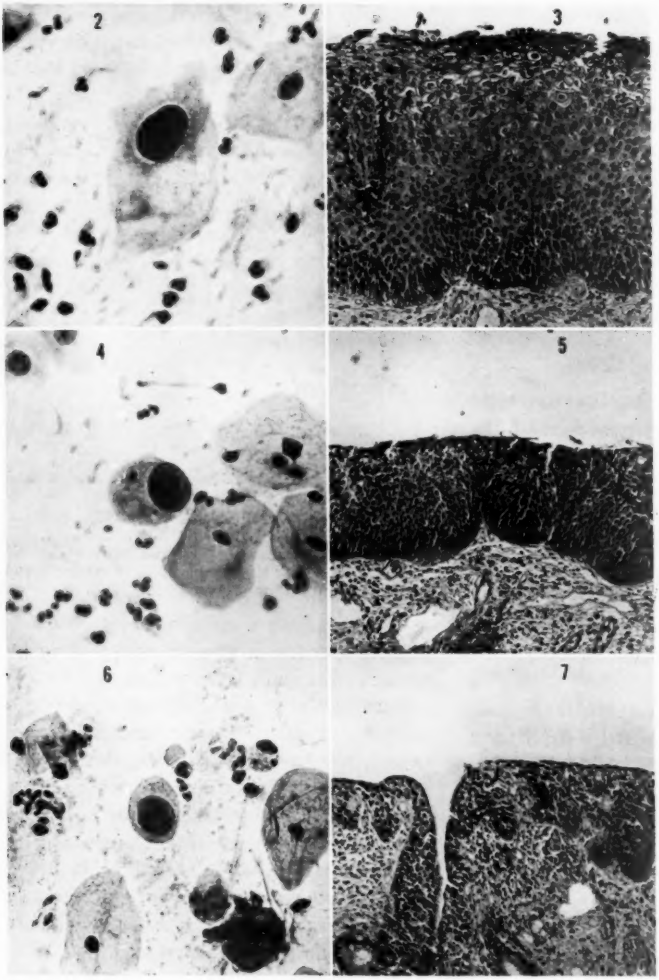

FIG. 2. Altered large cell of squamous type in dysplasia. The size, nucleoplasmic index, and cytoplasmic features are similar to those seen in the cells of the dysplastic reaction in Fig. 3 (from x500). FIG. 3. A dysplastic reaction involving a thick stratified squamous mucosa. There is prominent vacuolization of the cells and a surface change of parakeratosis (from x110). FIG. 4. Altered cell observed in dysplasia measuring 40 microns in its greatest dimension. The size, nucleoplasmic index, and cytoplasmic features are comparable to those observed in the cells of the dysplastic reaction in Fig. 5 (from x500). FIG. 5. A dysplastic reaction involving a thin mucosa made up of squamous-like cells. Compare thickness of mucosa with that observed in Fig. 3. This may represent dysplastic change in a mature metaplastic mucosa (from x110). FIG. 6. Altered cell observed in dysplasia measuring 25 microns in greatest dimension. The size, nucleoplasmic index, and cytoplasmic features are similar to those observed in some of the cells of the lesion depicted in Fig. 7 (from x500). FIG. 7. This reaction co-existed with that illustrated in Fig. 5. This change was observed more proximal and represents an atypical metaplasia with reserve cell hyperplasia (from x110).

other reactions having either larger or smaller cell types. Small cells as depicted in Figure 6, having a diameter of about 25 microns, were more commonly associated with a histopathological process as illustrated in Figure 7. The cell type was comparable in size to that observed in immature squamous metaplasia (25.9 ± 4.1 microns in mean diameter) and also had cytoplasmic features observed in the metaplastic cell.[20] The cell type was often associated with a variable number of smaller cells having features of reserve cells. The histopathological reaction also may co-exist with a reserve cell proliferation.

When considered in relation to the duration of pregnancy there was some variation in the size of the abnormal cells sampled. In women studied in the first trimester of pregnancy the mean cell area was 1204.6

± 537.9 square microns, in the second trimester the mean cell area was 1321.8 ± 691.4 square microns, and in the third trimester the mean area of the abnormal cells was 1706.2 ± 659.6 square microns. In general, lesions with a predominance of smaller cells were more common in the earlier stages of pregnancy while lesions made up of somewhat larger cells were more numerous in later stages of pregnancy.

Cell Shape

Of the abnormal cells surveyed 66.4 per cent had an isodiametric configuration while 33.6 per cent were nonisodiametric in form. The predominance of isodiametric forms was also observed in a previous study.[33] A total of 57.4 ± 16.5 per cent of the cells were polyhedral in shape, 32.9 ± 12.2 per cent were oval, 9.0 ± 5.3 per cent were round, 0.4 ± 0.7 per cent were elongate, 0.2 ± 0.3 per cent were caudate and 0.1 ± 0.3 per cent were otherwise irregular.

The configuration of the cells varied somewhat with the nature of the histopathological reaction and the duration of the pregnancy. Polygonal forms were more abundant in lesions made up of larger cells while oval and round cells were more numerous in patients having a lesion made up of smaller cells. Similarly in specimens from women in the first trimester of pregnancy there were a greater number of round or oval cells and a higher incidence of small cell lesions on histopathological study. In the later stages of pregnancy, the polygonal cells were more numerous as were histopathological lesions made up of larger and more differentiated cells. Caudate, elongate, and otherwise irregular configurations were uncommon as observed in previous studies dealing with cells arising in comparable reactions in the pregnant and nonpregnant woman.[33]

Cell Cytoplasm

As in most human cells a distinct cell membrane could not be identified at the periphery of the altered cells. The cytoplasm appeared in the form of a precipitate which varied in its density and staining reaction. The reaction was cyanophilic in 82.0 ± 11.5 per cent of the cells, eosinophilic in 15.3 ± 11.7 per cent of the cells and indeterminate in 2.7 ± 2.4 per cent of the cells. The staining reaction did not vary significantly with the histopathological process or the duration of pregnancy. In 20.1 ± 8.1 per cent of the cells there was cytoplasmic vacuolization. This was sometimes confined to the endoplasmic zone as in the cell depicted in Figure 2, while in other cells it was more diffuse.

Nuclear Size

The mean nuclear diameter in the abnormal cells was 16.3 microns and the computed mean nuclear area 209.9 ± 44.4 square microns. This value is higher than that observed in a previous study.[33] The explanation for this difference is not certain. The nucleoplasmic index which is a numerical expression of the nuclear size in relation to cell size NP Index = Nuclear area/Cell area − Nuclear area was computed to be 0.18 in the abnormal cells. The mean nuclear area varied with the stage of pregnancy. The mean nuclear area in specimens from women in the first trimester of pregnancy was 233.5 ± 53.6 square microns, in material from women in the second trimester of pregnancy the mean was 189.9 ± 42.1 square microns, and in specimens obtained during the third trimester of pregnancy the mean nuclear area was 208.6 ± 25.9 square microns. This was also reflected in the mean nucleoplasmic index which was 0.24 for abnormal cells derived from lesions in the first trimester, 0.16 for cells of reactions observed in the second trimester, and 0.13 for cells seen during the third trimester. In general, the nuclear mass was relatively large in cells from the earlier stages of pregnancy and relatively small in cells from reactions demonstrated

in later stages of pregnancy. This suggested that the lesions encountered earlier in pregnancy had a greater content of primitive cells.

Nuclear Shape

The configuration of the nucleus was more or less related to that of the cell, being oval in 79.7 ± 9.3 per cent of the cells, round in 19.4 ± 9.1 per cent of the cells, irregular in 0.5 ± 0.5 per cent of the cells and flat in 0.4 ± 0.2 per cent of the cells. The latter form was observed in elongate cells.

Nuclear Structure

The chromatinic material of the nucleus was hyperchromatic and basically finely granular in 90.4 ± 6.8 per cent of the cells, hyperchromatic and coarsely granular in 3.7 ± 0.78 per cent, and hyperchromatic with translucent or opaque nuclear content in 5.9 ± 4.6 per cent of the cells. While 91.2 per cent of the cells were mononucleate, 8.8 per cent contained more than one nucleus. In some nuclei there were distinct chromocenters and a rare nucleus contained a distinct acidophilic nucleolus. None of the cells had a macronucleolus. Although the nuclear structure in some cells might be compatible with an impending prophase there were no cells in metaphase or anaphase. Rare nuclear forms were vacuolated. These were observed in cells with associated cytoplasmic vacuolization.

Biologic Nature

All of the 28 patients included in this study had both cellular and histopathologic evidence of a dysplastic reaction in the uterine cervix during pregnancy. In 14 patients the cellular sample obtained at the time of the initial postpartum examination was within normal limits. Similarly one or more subsequent cellular samples were within normal limits. Although it was impossible to exclude the possibility that biopsy in itself may have altered the course of the lesions, there was no evidence to indicate that the lesion was removed *in toto* at the time of biopsy. In these women a reaction known to be present during pregnancy was not evident in the postpartum period as determined by cellular samplings.

Of the 14 patients known to have had dysplasia during pregnancy and in the postpartum period, five subsequently had cellular studies which were within normal limits after intervals of four months, six months, one year, one year, and two years. Subsequent cellular samplings were also within normal limits. While biopsy may have influenced the course of these lesions there was no evidence to indicate that punch biopsy removed any of these lesions inadvertently.

In six additional patients the reaction which was present during the pregnancy and in the postpartum period persisted for varying periods of time. The lesion persisted in one patient for the five years of follow up despite biopsy on seven occasions and 38 cellular samplings. In five patients the lesion persisted only to be removed by biopsy after periods of six months, one year, one year, three years, and three years. In four of the patients the change persisted without significant progression until it was inadvertently removed by punch biopsy. In one woman, the cellular evidence indicated progression and the lesion was deliberately removed by sharp knife conization. On histopathologic study the lesion was considered to be a marked dysplastic reaction, however an early developmental stage of cancer was not entirely excluded.

In two patients, subsequent studies were more compatible with *in situ* cancer. This was first recognized seven months after delivery in one case and four years after delivery in another patient. Hysterectomy was done in both patients.

One remaining patient was lost to follow up although the lesion was present in the postpartum period on the basis of cellular findings.

Cells in Relation to Course

In comparing cellular specimens from patients having a persisting change with those obtained from patients who did not have evidence of the lesion in the postpartum period there was only one significant difference. Counts of abnormal cells were higher in the initial specimen from patients having a persistent or progressive change. The lesion persisted in the postpartum period in two of 12 patients having counts of 0-199, in one of three patients having counts of 200-399, in four of six patients having counts of 400-599 and in seven of seven women having counts in excess of 600 per slide. It was impossible to determine the fate of the lesion on the basis of the appearance of the cells in either the initial cellular or histopathological specimen although in general the more severe reactions were more likely to persist and the less pronounced reactions were more likely to disappear after pregnancy. The appearance of the initial specimens from those patients who were ultimately proved to have *in situ* cancer was not significantly different from the remaining cases.

Discussion

The lesions designated as dysplasia in this study are of importance because this reaction may be demonstrated prior to or actually co-exist with *in situ* or invasive cancer. It may exist alone in other cases, persisting for varying periods of time or ultimately undergoing regression. The basic derangement in the stratified squamous mucosa may be a defect in maturation. As a result some of the cells are prematurely displaced upwards in the epithelium where they are conspicuous because of the large size of their nuclear mass and for certain other abnormalities. A comparable change is observed experimentally after the application of certain carcinogens to skin or mucous membrane. Some but not all of the dysplastic lesions are composed of cells which are actively proliferating as evidenced by the presence of normal or abnormal mitosis. It is not the purpose of this paper to describe the histopathologic features in detail. In the literature, there are excellent descriptions of the lesion in both the pregnant[2, 6, 24] and nonpregnant woman.[16, 21, 29]

Similar or related changes have been described in the literature under a varied terminology which adds to the confusion concerning the reactions. Some of the more common terms which have been applied include atypia,[18] benign atypia,[1, 15] simple atypical epithelium,[12] irregular,[7, 34] and increasingly atypical epithelium,[13] squamous metaplasia with atypicality,[16] anaplasia,[21, 35] precancerous metaplasia,[3] dissociated intraepithelial anaplasia,[27] dysplasia,[5, 6, 28, 33] basal cell hyperplasia,[9, 11, 26] or hyperactivity;[9, 10] dyskaryosis,[27] leukohyperkeratosis[30] and leukoparakeratosis.[30] There are in addition, numerous lesions depicted in the medical literature as examples of *in situ* cancer whose changes are similar to those categorized as dysplasia in this study.

The occurrence of dysplasia or a morphologically related change in a predominantly nonpregnant population has been described previously. On the basis of histopathological studies, the prevalence has been reported to be 1.2 per cent,[21] 2.0 per cent,[11] 2.3 per cent,[18] 3.1 per cent, [10] and 3.2 per cent.[29] Although more varied, the values described for the occurrence of the reaction in the gravid woman are somewhat higher. This has been reported to be 6.0 per cent,[22] 6.8 per cent,[23] 14.7 per cent,[8] and 30.0 per cent.[2] Unfortunately the reported occurrence of the lesion in pregnant and nonpregnant patients is not neces-

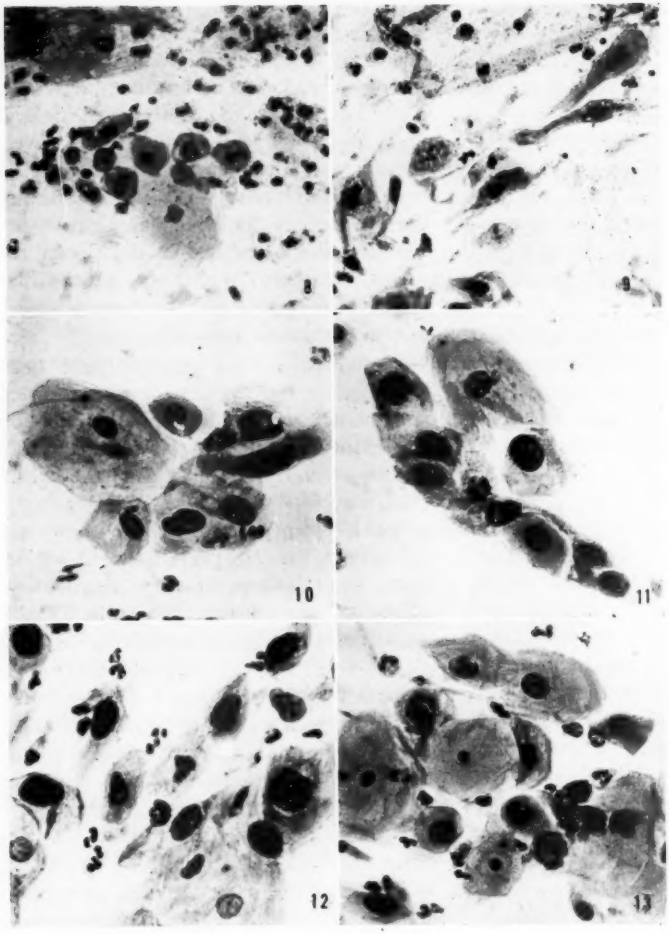

FIG. 8. Small cells with pyknotic nuclei and acidophilic cytoplasm originating in parakeratosis covering dysplastic reaction as observed in Fig. 3 (from x500). FIG. 9. Bizarre cell forms including elongate, caudate, and irregular configurations which probably originate from surface change in dysplasia (from x500). FIG. 10. Dysplasia in pregnancy. Abnormal cell forms with variable nuclear enlargement occurring in relation to a squamous cell and a small cell originating in squamous metaplasia (from x500). FIG. 11. Dysplasia in pregnancy. Abnormal cell forms of variable size with nuclear enlargement. One cell in the center is undergoing karyorrhexis. Nuclear vacuolization is present in one cell (from x500). FIG. 12. Dysplasia in pregnancy. Both polygonal and oval or ellipsoidal cells are represented together with free nuclei. Nuclear configuration is more often more or less oval in oval cells and round in isodiametric cells (from x500). FIG. 13. Dysplasia in pregnancy. Smaller abnormal cells are represented in this illustration. Some are approaching round shapes. There is nuclear enlargement and hyperchromasia. Elsewhere in the specimen there were larger abnormal cells (from x500).

sarily comparable. Differences in (1) selection of the sample (2) the size of the sample (3) the composition of the population studied and (4) in the histopathologic criteria employed in establishing the diagnosis might account for the differences in the described occurrence rates. The evidence at least suggests that the reaction is more commonly observed in the uterine cervix of the pregnant woman.

The cellular sample collected from the pregnant patient similarly may indicate the presence of an abnormality. Some degree of cellular abnormality has been reported in 0.7 per cent,[32] 2.0 per cent,[14] 2.5 per cent,[19] 3.6 per cent,[17] 9.2 per cent,[25] and 14.1 per cent,[4] of the specimens examined. Inasmuch as several different types of sampling were used in these studies there is a considerable difference in the number of demonstrable changes although other factors are also involved. In only a few studies is there information regarding the frequency of dysplasia on the basis of cellular

studies. This has been reported to be 0.15 per cent,[31] 0.5 per cent,[5] and 3.0 per cent in the present study. The occurrence rate of 3.1 per 1,000 observed in a pregnant population is about double the occurrence rate demonstrated in this institution for dysplasia in nonpregnant patients. In correlating cellular and histopathological changes it is essential to have detailed knowledge of both the cellular sample and the tissues from which the cells are derived. In the process referred to as dysplasia several different reactions may occur simultaneously in the uterine cervix resulting in a heterogeneous population of cells.

The population of abnormal cells includes cells originating in the abnormality of the stratified squamous mucosa depicted in Figure 3. Over the surface of this there may be a change designated as parakeratosis giving rise to cells depicted in Figure 8 or in other instances on excessive formation of keratin from which anucleate squames originate. Also represented in the abnormal cell population may be cells originating from a somewhat thin mucosa whose altered cells have some of the features of squamous cells. This reaction illustrated in Figure 5 may be developing in a site of mature squamous metaplasia. The endocervical mucosa and its sub-columnar cells may have a different type of response. This may be a proliferation of the so-called "reserve" cells or an immature type of squamous metaplasia whose nuclei are abnormally large for this cell type. Such a change is illustrated in Figure 7. These three basic reactions account for the various abnormal cell forms in the cellular preparation.

One or more of these basic reactions may exist in the uterine cervix although one is usually dominant. There is evidence to suggest that the reaction is primarily in the endocervical mucosa in the early stages of pregnancy and in the stratified squamous mucosa in later stages of pregnancy and in the nonpregnant patient.

The cells are predominantly isodiametric in the presence of dysplasia. These are usually polygonal in form as are some of the cells in Figures 10-13. The configuration is in part related to the cell type and to the pressures exerted on the cells in the tissues. Round or oval cells may also be observed as indicated in Figures 12 and 13. Such forms are more numerous in lesions having reactions in what is thought to be pre-existing metaplastic epithelium. Their form is related to the unequal pressures exerted on the cells in the mucosa. Elongate, caudate, or otherwise irregular forms are not common but they are observed in some lesions. These are illustrated in Figure 9. Their most likely site of origin is the abnormal surface reaction overlying altered stratified squamous mucosa. The configuration of the elongate and caudate forms may be related to the presence of a linear fibril which is probably a remnant of the fibrillar apparatus known to be involved in keratinization.

The nuclei of the cells originating from a dysplastic reaction are abnormally large in relation to the cell type. Since all cells are not equally affected there is considerable variation in the size of the nuclear forms. The nuclear membrane may be wrinkled, indicating degeneration. The chromatinic material may be quite delicate and only slightly hyperchromatic. These nuclear forms as depicted in Figures 10-13 are more reminiscent of the interphasic nucleus insofar as the structure is concerned. Other nuclear forms have a more hyperchromatic and coarse chromatinic material either with or without larger chromatin aggregates called chromocenters. Varying degrees of nuclear translucency or opacity may be observed particularly in cells undergoing degeneration as indicated in Figures 9 and 11. While most cells are mononucleate, binucleation and less com-

monly multinucleation may be observed.

The management of the patient with dysplasia depends in large part on the histopathological findings at biopsy. This may or may not give information as to the probable fate of the lesion. Since in some lesions there may be progression and in other lesions a regression in the postpartum period, the initial biopsy may not provide information as to the outcome. On reviewing the initial cellular samples there were no morphological changes which correlated with the course of the lesion. Of the various features evaluated, only the number of abnormal cells represented in the sample could be correlated with the presence or absence of the change in the postpartum period. Neither this nor any of the cellular features evaluated gave information as to which patients would ultimately be proved to have carcinoma *in situ* or would have progression of their abnormality. Obviously in dealing with a dysplastic reaction, multiple cell studies and multiple biopsies can be used to determine the outcome of the lesion.

These observations in no way imply that cellular studies should be used in place of biopsy. Cellular data should be used to complement the information gained by biopsy and often can be used to advantage in planning biopsy in a patient whose lesion is not apparent on inspection of the uterine cervix, or in indicating the presence of a process which may not be apparent in the initial biopsy specimen.

Summary

Some degree of cellular abnormality was observed in 6.2 per cent or 58 of 930 pregnant women studied by means of cellular samples. In 28 or 3 per cent of the women studied there was cellular evidence of dysplasia subsequently confirmed on histopathologic study. The occurrence of any cellular abnormality and the occurrence of cellular changes of dysplasia was related to the parity of the patient. Cellular abnormalities were more common with increasing parity.

Cellular samples from patients with dysplasia contained a variable number of altered cell forms. These had a mean diameter of 42.4 microns and a mean area of 1407.7 ± 648.19 square microns. Isodiametric forms were more common accounting for 66.4 per cent of the cells while 33.6 per cent were nonisodiametric. The cytoplasmic staining was cyanophilic in 82.0 per cent of the cells, eosinophilic in 15.3 per cent and indeterminate in 2.7 per cent. The mean nuclear area was 209.9 ± 44.4 square microns and the average nucleoplasmic index 0.18. The nucleus was usually round or oval in form with a finely granular structure in 90.4 per cent of the cells, a coarsely granular structure in 3.7 per cent of the cells and constituted a translucent or opaque mass in 5.9 per cent of the cells. Cellular changes reflected the structure of the lesions of origin, varying in relation to the stage of pregnancy and the type of reaction which was predominant.

Of the 28 women with dysplasia, 14 did not have cellular evidence of the change in the postpartum period. Of the 14 patients with demonstrable changes in the postpartum period, five subsequently had negative studies, six had persistence for varying periods, two were subsequently proved to have *in situ* cancer and one was lost to follow up studies.

On reviewing the cellular samples in relation to the ultimate outcome, there was no significant correlation between morphology in the initial specimen and outcome. In general, however, there were more abnormal cells in the initial samples from women having lesions which were evident in the postpartum period.

Résumé

930 femmes gravides on été examinées par des frottis cytologiques du col utérin. 58 femmes

ou 6.2% ont montré des cellules anormales et chez 28 femmes ou 3%, les cellules ont montré des signes évidents de dysplasie confirmée ultérieurement par des biopsies. On a comparé la fréquence des cellules anormales et des modifications cellulaires de dysplasie avec la parité des malades. Les anomalies cellulaires ont été plus fréquentes avec la parité croissante.

Les frottis provenant de femmes ayant une dysplasie ont contenu un nombre variable de cellules modifiées. Celles-ci ont un diamètre moyen re 42.4 microns et une surface moyenne de 1407.7 ± 648.19 microns carrés. Les formes isodiametriquez ont été les plus nombreuses, allant jusqu' à 66.4% des cellules, tandis que 33.6% ont été non-isodiamétriques. La réaction tinctoriale cytoplasmique a été basophile dans 82.0% des cellules, acidophilique dans 15.3% et indéterminée dans 2.7%. Les noyaux ont montré une surface moyenne de 209.9 ± 44.4 microns carrés et l'index mucléoplasmique moyen a été de 0.18. Le noyau a montré une forme arrondie ou ovale avec une structure granuleuse fine dans 90.4% des cellules, une granulation plus grossière dans 3.7% et a formé une mass translucide ou opaque dans 5.9% des cellules. Les modifications cellulaires ont réflété la structure de la lésion de base, avec des variations en rapport avec l'âge de la grossesse et le type de réaction prédominante.

Parmi les 28 femmes ayant montré une dysplasie au cours de la grossesse, 14 n'ont plus montré des signes cytologiques de ces modifications au cours du post-partum. Parmi les 14 femmes avec persistance des altérations cytologiques dans le postpartum, 5 femmes ont eu des frottis négatifs ultérieurement, 6 ont continué à présenter les altérations pendant des périodes variées, et chez deux on a pu identifier ultérieurement un cancer in situ. Une femme été perdue de vue.

En réexaminant les frottis par rapport à l'évolution ultérieure, on n'a pas pu trouver de correlation signifiante entre la morphologie du premier frottis et l'évolution ultérieure. Cependant, on a constaté en général un nombre plus élevé de cellules anormales dans les premiers frottis chez les femmes où la lésion a pu être retrouvée dans la période du post-partum.

Zusammenfassung

Gewisse zelluläre Abnormitäten wurden in 6.2% oder in 58 Patientinnen in einem Patientengut von 930 schwangeren Frauen beobachtet, die mittels zytologischer Ausstriche untersucht wurden. In 28 Patientinnen oder in 3% der untersuchten Fälle war der zytologische Befund "Dysplasie" erhoben worden, der auch im Anschluss histologisch bestätigt wurde. Das Auftreten von Zellabnormitäten im allgemeinen und das Auftreten von Zellveränderungen, die auf Dysplasie hinweisen, im besonderen, wurden zum Alter der Patientin in Beziehung gesetzt. Zellabnormitäten sind häufiger mit erhöhter Parität.

Das Zellmaterial von Patientinnen mit Dysplasie enthielt in verschiedenen Mengen veränderte Zellformen. Der Mittelwert der Durchmesser der veränderten Zellen war 42.4 μ und der Mittelwert der Zellfläche war 1407 ± 648.19 μ^2. Isodiametrische Zellformen wurden in 66.4% angetroffen, während in 33.6% keine isodiametrischen Zellformen vorhanden waren. Die Zellfärbung war cyanophil in 82%, eosinophil in 15.3% und unklar in 2.7%. Der Mittelwert der Kernfläche war 209.9 ± 44.4 μ^2 und der Durchschnittswert des Kern-Plasma-Indexes war 0.18. Der Kern war gewöhnlich rund oder oval geformt, mit fein granulärer Struktur in 90.4% der Zellen, mit grob granulärer Struktur in 3.7% der Zellen und bestand aus einer durchscheinenden oder nicht durchscheinenden Masse in 5.9% der Zellen. Die Zellveränderungen spiegelten die Struktur der Ursprungsläsion wider und zwar in verschiedenen Graden, je nach dem Alter der Schwangerschaft und je nach dem Typ der vorherrschenden Epithelreaktion.

Von den 28 Patientinnen mit Dysplasie hatten 14 während der Postpartum Periode keine Anzeichen mehr von der Zellveränderung. Von den übrigen 14 Patientinnen mit noch vorhandenen und nachweisbaren Veränderungen während der Postpartum Periode zeigten 5 später negative Befunde, 6 zeigten Weiterbestehen der Veränderungen während verschieden langer Zeiträume, in 2 Fällen wurden später Carcinomata in situ nachgewiesen, während eine Patientin nicht nicht nachuntersucht werden konnte.

Bei der Durchsicht des Zellmaterials in Beziehung zum endgültigen Befund konnte man keine signifikante Korrelation zwischen der Morphologie im ersten Zellpräparat und dem endgültigen Abschlussbefund finden. Im allgemeinen jedoch waren mehr abnormale Zellen in den ersten Präparaten derjeniger Patientinnen zu finden, die dann auch noch in der Postpartum Periode abnormale Zellen in den Präparaten zeigten.

Resumen

930 gestantes han sido estudiadas desde el punto de vista citológica, con objeto de evaluar los cambios de tipo displastico que ocurren en el cérvix uterino durante el embarazo. En 58 de los 930 casos (6.2%) se pudo observar algún grado de anormalidad celular. En 28 casos (3%) habia evidencia celular de displasia, confirmada posteriormente por histopatologia. Se relacionó, asimismo, la presencia de algun tipo de alteración celular y de cambios de tipo displastico con la paridad de la paciente, encontrando que las anomalias celulares son mas frecuentes conforme la paridad aumenta.

Los frottis de pacientes con displasia contienen un número variable de formas celulares alteradas. Se encontró en éstas un diámetro medio de 42.4 micras y una superficie media de 1407.7 ± 648.19 micras cuadradas. Fueron mas comunes las formas isodiamétricas (66.4%) que las anisodiamétricas (33.6%). La coloración protoplásmica fue de tipo basófilo en 82.0% de las células, acidófilo en 15.3%, e indeterminado en 2.7%. El área nuclear media fué 209.9 ± 44.4 micras cuadradas y el valor medio del índice núcleo/protoplasma de 0.18. El núcleo fué generalmente de forma redonda u oval, con una estructura finamente granular en 90.4% de las células, de gránulos gruesos en 3.7%, y en forma de una masa translúcida u opaca en 5.9%. Los cambios celulares reflejaron la estructura de las lesiones, variando en relación con el estadio del embarazo y el tipo de reacción que era predominante.

De las 28 mujeres con displasia, 14 no presentaron cambios celulares evidentes en el postpartum. De las restantes 14, en las que se apreciaba algún cambio, presentaron resultados negativos en ulteriores estudios 5 casos; en 6 las alteraciones celulares persistian a intervalos variables, en 2 se demostró posteriormente la existencia de un carcinoma in situ, y en 1 caso los estudios posteriores no fueron concluidos.

Relacionando los hallazgos celulares en distintas muestras y el resultado final, no se encontró correlación evidente entre morfologia en la muestra inicial y resultado final. En general, sin embargo, habia mayor número de células anormales en las muestras iniciales de mujeres que tenian lesiones evidentes en el postpartum.

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Erratum

The captions of the photographs submitted by Dr. Jacqueline Mouriquand for her paper in Volume III, No. 3, pages 451-454, 1959 were inadvertently omitted. The captions of the figures are as follows: FIG. 1. Multinucleated large cell in a squamous cell carcinoma; FIG. 2. Multinucleated large irradiated cell; FIG. 3. Malignant cells; FIG. 4. Irradiated cells: no carcinoma (Patient followed over a period of three years); FIG. 5. Squamous cell carcinoma; FIG. 6. Irradiated squamous cell carcinoma.

Symposium on Probable or Possible Premalignant Cervical Lesions

See Table of Contents on pages 2 and 3 for listings in this issue.

This Symposium will be continued in the March-April Issue. The following is a list of forthcoming topics:

II. (Continued) Ectopy, Ectropion and Epidermization

Colposcopy of Ectopy, Ectropion and Epidermization

Colpomicroscopy of Ectopy, Ectropion and Epidermization

Clinical Viewpoint: Management of Ectopy, Ectropion and Epidermization

Interrelationship: Ectopy-Ectropion-Epidermization and Cervical Carcinoma

III. Leukoplakia

Histomorphology of Leukoplakia

Histochemistry of Leukoplakia

Exfoliative Cytology of Leukoplakia

Animal Experiments

Colposcopy of Leukoplakia

Colpomicroscopy of Leukoplakia

Clinical Viewpoint on the Management of Leukoplakia

Interrelationship: Leukoplakia and Cervical Carcinoma

IV. Reserve Cell Hyperplasia, Basal Cell Hyperplasia and Dysplasia

Histomorphology of Reserve Cell Hyperplasia, Basal Cell Hyperplasia and Dysplasia

Histochemistry of Reserve Cell Hyperplasia, Basal Cell Hyperplasia and Dysplasia

Exfoliative Cytology of Reserve Cell Hyperplasia, Basal Cell Hyperplasia and Dysplasia

Fluorescence Microscopy of Hyperplasia in Gynecological Cytodiagnosis

Animal Experiments

Reserve Cell Hyperplasia, Basal Cell Hyperplasia and Dysplasia

Colpomicroscopy of Reserve Cell Hyperplasia, Basal Cell Hyperplasia and Dysplasia

Clinical Viewpoint on the Management of Reserve Cell Hyperplasia, Basal Cell Hyperplasia and Dysplasia

Interrelationship: Reserve Cell Hyperplasia, Basal Cell Hyperplasia, Dysplasia and Cervical Carcinoma

Symposium on Probable or Possible Premalignant Cervical Lesions

Histomorphology of Cervicitis and Endocervicitis

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THE INFLAMMATION of the cervix uteri, also called cervicitis, holds a central position as a possible initial point of subsequent inflammatory changes of the female genital organs. This fact can be explained by the topography and the physiological function of the cervix uteri. Ascending inflammations higher in the genital tract, as well as pathologically resulting conditions of the portio vaginalis uteri (ectocervix), of the vaginal walls and of the vulva, may result from inflammatory changes of the endocervix. Most of the inflammations of the cervix are caused by exogenous irritations. To some extent the agents causing an inflammation work via the blood stream.

A classification of causes and morphological symptoms is suitable for a summarizing description of cervicitis.

I. Causal Pathogenesis

A. Infections
   1. Gonorrhea, mixed flora
   2. Typhus fever, scarlet fever, cholera
   3. Tuberculosis and actinomycosis
   4. Protozoa (for instance Trichomonas vaginalis, Donne)
   5. Rare worms (pinworm)

B. Chemical irritations
   1. Disinfectants (irrigations)
   2. Anticonceptional means
   3. Caffeine and nicotine

C. Mechanical irritations
   1. Foreign substances of every kind (masturbation)
   2. Occlusive pessary with pin, intra-uterine pessary
   3. Bases for medications (for instance talcum powder)

D. Diverse causes
   1. General diseases
   2. Psychic exhaustion
   3. Constitutional factors, such as hypoplasia of genitals, infantilism, hypofunction of the ovaries, weakness of the connective tissue

II. Formal Pathogenesis (Morphological Psycho-Visual Image)

A. Acute inflammation
   1. Dull swelling, so-called alterative inflammation of the epithelia of the cervix
   2. Serous inflammation, prototype catarrh: increased production of mucus with increased proliferation and desquamation of epithelia of the cervix
   3. Fibrinous and fibrino-purulent inflammation (pseudomembranous inflammation)
   4. Purulent inflammation such as abscess, phlegmon, diffuse suppuration and local periglandular inflammation

B. Chronic inflammation
   1. Lympho-plasmacellular
   2. Proliferative-fibroplastic
   3. Ulcerative
   4. Polypous and cystic
   5. Atrophying inflammation
   6. Metaplastic inflammation (anaplasia)

C. Specific inflammation
   1. Proliferative and ulcerous tuberculous
   2. Ulcerative typhous
   3. Basket (Drusen) forming inflammation with actinomycosis.

The catarrhal inflammation is frequent. The catarrh is mainly a serous inflammation with increased proliferation and desquamation of epithelia. The purulent inflammation is important. Prototype: the gonorrhea of the cervix. The typical purulent cervicitis is at first an inflammation at the surface of the epithelium. All areas of the mucous membrane, also the epithelial crypts, are included in the inflammatory process. If the process persists, there occurs fusion of the surface-cells, and later on

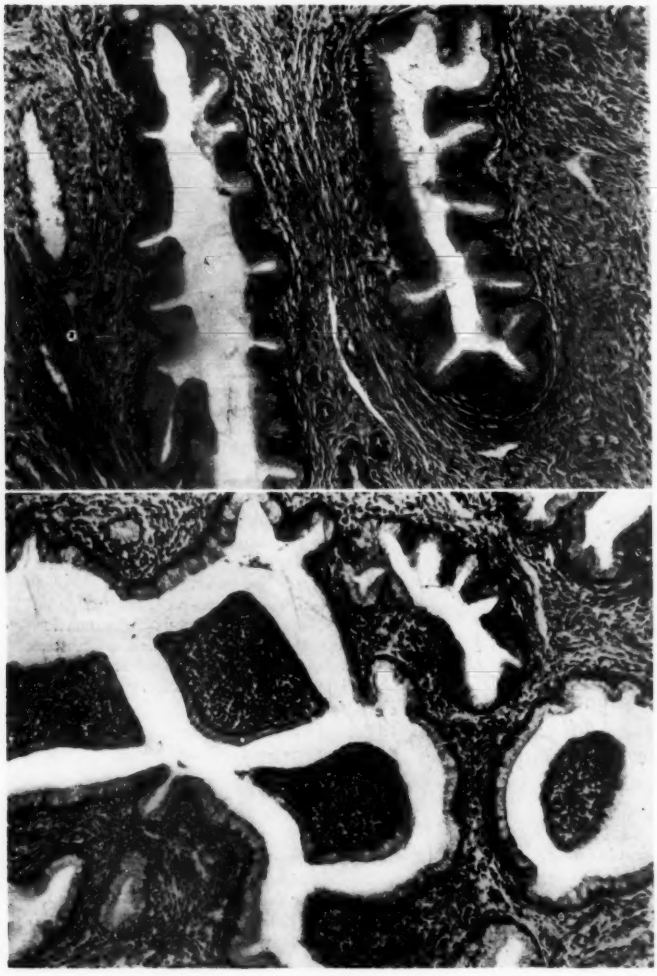

FIG. 1. (Top) Normal epithelium of the cervix. FIG. 2. (Bottom) Chronic cervical inflammation with papillar proliferation.

even ulceration and infiltration of the interstitial tissue with pyocytes.

Polyps originate in the cervix nearly always as a resulting condition of chronically relapsing inflammations. The polyp can be papillar, glandular or tubular. Mostly it is compact and shows a smooth surface, but often it is very fissured. Nearly always it is eroded, infected and chronically inflammatorily changed or even suppurated. So-called epidermization of the surface is frequent. Polyps are often stalked.

Formation of cysts of cervical glands is often found after inflammatorily-caused occlusion of the glandular lumina. In the cervix the mechanism of the occlusion cannot be caused by the overgrowing of pavement epithelium as we know it from the ovula nabothi of the ectocervix. The cysts mostly contain mucus; the epithelium

of the cysts becomes cubic or flattened by pressure. Runge showed encapsulated virulent gonococci in such cysts, after clinical cure. Cystic and atrophying inflammation, both more frequently present in the climacterium and menopause than during the period of puberty, similarly injure the normal function of the cervix for awhile.

The so-called metaplasic (anaplastic) cervical inflammation can follow from every chronic inflammatory irritation, which is associated with a permanent or changing acidification of the alkaline milieu of the cervix. In the course of many cell generations there occurs in the cervix itself a "transformation" of cervical epithelium into lower degrees of epithelial differentiation. Here a chronic intra-epithelial inflammation is always demonstrable.

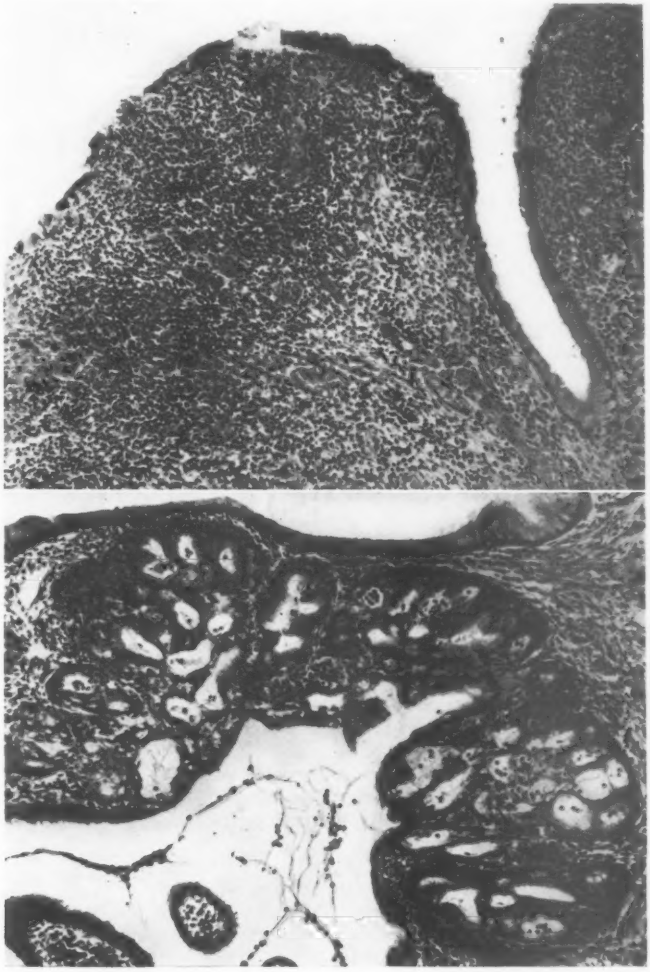

FIG. 3. (Top) Purulent cervical inflammation with superficial epithelial defects. FIG. 4. (Bottom) Cervical inflammation with beginning metaplasia of the epithelium.

FIG. 5. (Top) Cyst-forming inflammation of the cervix uteri. FIG. 6. (Bottom) Surface of a cervix polyp with erosion, inflammation and bleeding.

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CARLO SIRTORI
Milano, Italy

CERVICITIS due to Trichomonas is characterized by chromatin dispersion, thickening of the nuclear membrane, cytoplasmic vacuoles and loss of the normal stratification of the epithelium.

Nonspecific cervicitis shows: hyperplasia of the basal cells, often followed by gland epidermization or marked keratinization of the ectocervical squamous epithelium.

In the basal layers of primary syphilomas (eight cases observed) tripolar atypical mitoses and irregular stratification of the epithelium are often found.

Discussion

Hans F. Bettinger, Melbourne, Australia: I have to ask Korte one question. Are metaplasia and anaplasia really synonymous?

Closing Remarks

Wolfgang Korte: I thank Bettinger for his hint. I should have added an explanation. In our usage of terms, metaplasia is the general larger term. I understand anaplasia to mean the transformation or transition into a lower degree of differentiation of the same tissue, for instance cylindrical epithelium into pavement epithelium. Prosoplasia is the transition into a higher degree of differentiation. On the cervix, I do not know a prosoplastic inflammation.

Histochemistry of Cervicitis and Endocervicitis

JEAN BERGER
Basel, Switzerland

WE HAVE studied only six cases which could colposcopically be designated as localized or generalized colpitis by means of the methods described in and listed in "Histochemistry of Ectopy, Ectropion and Epidermization." We have not been able to prove a significant difference from the normal epithelium. The demonstration of DNA and RNA, as well as of the acid phosphatase and phosphoamidase, have been the same as in normal cells, qualitatively and in regard to the distribution within the cells. Differences from the normal epithelium and from the atypical conditions can be recognized with the usual histological criteria.

Summarizing, we may say that in the diagnosis of the epithelial changes in cervicitis the histochemical procedures do not render any additional information and that the classical histological methods give sufficient orientation. For this reason we renounce the display of microphotographs.

ALVAN G. FORAKER
Jacksonville, Florida, U.S.A.

THE REACTIONS of cervicitis involve the epithelium, and the inflammatory process. Inflammation frequently has a stimulating effect upon the epithelium. In the cervix, this is best known in the effect of Trichomonas vaginitis producing atypical cells as observed in smears and sections. These epithelial reactions are secondary, and it is unlikely that intrinsic histological or histochemical differences are found in atypical epithelium stimulated by inflammation, as compared to primary agnogenic cellular changes. The histochemistry of inflammation has not been studied in extracervical lesions as much as it should. Some years ago we[1] found marked evidence of dehydrogenase activity in foreign body giant cells surrounding cholesterol crystals in chronic salpingitis. In the cervix, we[2] observed considerable evidence of dehydrogenase activity in lymphoid follicles appearing in intense chronic cervicitis. Inflammatory cells in general showed a somewhat lesser degree of evidence of dehydrogenase activity than did adjacent endocervical columnar epithelium.

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Exfoliative Cytology of Cervicitis and Endocervicitis

EMMERICH VON HAAM
Columbus, Ohio, U.S.A.

THE FREQUENCY of cervicitis and endocervicitis in women above the age of 25 is well reflected in the many inflammatory changes encountered in their vaginal smears. At first, only few pathologists paid much attention to the diagnostic value of these inflammatory cells, and it was the general feeling that they represented rather a nuisance value interfering with the recognition of atypical or malignant cells. However, the proper evaluation of inflammatory changes in the vaginal smear has become to us a very valuable indication concerning the presence of inflammatory changes in the cervix which are often symptomless except for a slightly increased vaginal discharge.

Inflammatory changes in the cervix can be recognized in the smear prepared from vaginal aspiration, but they are better studied in smears prepared by scraping the cervical mucosa including the area of the external os after visualization of it through a nonlubricated speculum. The inflammatory cells are either diffusely scattered over the slide or can be found aggregated in small clumps which often contain a center of necrotic epithelial cells.

Inflammatory changes of the epithelial cells themselves can be recognized by the appearance of vacuoles and perinuclear halos in the cytoplasm and a pronounced degree of eosinophilia or acidophilia among the epithelial cells, including the parabasal

cells. The nuclei of superficial vaginal epithelial cells lose their compact pyknotic appearance and are pale and vesicular. Often binucleate superficial cells can be found. Basal and parabasal cells may be increased, pointing to an ulcerative process.

We are classifying the degree of the inflammatory changes by estimating the number of inflammatory cells in relation to the number of vaginal epithelial cells, evaluating the inflammatory changes noticeable in the epithelial cells and examining the bacterial flora present on the smear. We interpret these inflammatory changes as compatible with or suggestive of "mild," "moderate," or "severe" cervicitis. The specific type of chronic cystic cervicitis can be recognized from the vaginal smear by large amounts of basophilic mucus on the slide. Inflammatory cells may or may not be present in some of these cases. Granulomatous endocervicitis produces much less mucus, but clumps of large and swollen endocervical cells can be noticed among the white and red blood cells. If we find pronounced differences in the presence of inflammatory cells between the vaginal aspiration smear and the cervical scraping smear we usually consider this an argument against the presence of a severe endocervicitis. For this reason both types of smears should be studied in order to avoid an erroneous interpretation.

EKKEHARD KOFLER, HANS KREMER
AND RUDOLF ULM
Vienna, Austria

THE CYTOLOGICAL changes in endocervicitis and cervicitis are identical. In the endocervical smear one finds, according to the severity of the inflammation, highly degenerated, altered cylindrical cells. In addition, numerous inflammatory cells appear, such as leukocytes, lymphocytes and plasma cells, which are frequently lying in clusters. Moreover, one often sees clumps of bacteria and trichomonads and large amounts of mucus. The most sensitive part of the cylindrical epithelial cells to inflammatory irritations is the cytoplasm. It rapidly loses its sharp borders and cytolysis takes place, leaving naked nuclei behind. Sometimes the nuclei in the undamaged cylindrical cells may have an elongated shape and thus remind one of the so-called "snake cells." Usually the nuclei in the cylindrical cells, altered by inflammation, are larger than normal. Their chromatin appears homogenous. Sometimes, however, one can observe a certain condensation of chromatin on the nuclear rim, making the nuclear outline appear very pronounced. Frequently, the chromatin is clumped or lies in cords. Such a picture can, in extreme cases, simulate an irregularity of the chromatin. In addition to the described nuclear changes, formation of vacuoles in the nucleus may take place. The benign, purely inflammatory-degenerative character of the process is clearly documented by the uniformity of cells and nuclei and is confirmed by the lack of pronounced irregularities of the chromatin.

GUILLERMO TERZANO
Buenos Aires, Argentina

ACUTE AND CHRONIC cervicitis are inflammatory processes and therefore the picture of inflammation dominates the background of their smears.

Abundant mucus, numerous polymorphonuclear leukocytes, lymphocytes, pyocytes (more in acute processes), erythrocytes (in scrapings of the cervix and even in vaginal aspirations), and histiocytes of the most varied shape and size are usually observed. Bacteria, cocci or parasites complete the picture.

Extensive desquamation of squamous and columnar epithelial cells, singly and/or in clusters, sheets, polypoid formations, pearl-like groups, etc., is commonly found.

In 3.2 per cent of our cases (55 out of 1693) no abnormal cytology was found in thoroughly screened smears. Abnormal cytology was classified in our series as Class II (1,530 cases or 90.3 per cent), Class III (102 cases or 6.02 per cent) and Class IV (6 cases or 0.36 per cent). In this last group, we have mistaken abnormal cells for cancer cells (false positive diagnosis).

By abnormal cytology we mean:

1. anisocytosis and poikilocytosis.
2. bizarre cells. Pseudospindle cells are the most frequently seen, but actual spindle-shaped and tadpole cells are not exceptional in smears of cases with cervicitis.
3. abnormal eosinophilic cytoplasm.
4. vacuolization, especially large single vacuoles which may be pseudophagocytosis.
5. nuclear alterations: lobulation, pyknosis or fragmentation of the nucleus. Multinucleation. Enlarged nuclei that may show rather coarse granular chromatin or even some hyperchromasia. (When inflammation reaches the parabasal zone of the epithelium and abnormal parabasal cells appear in smears, it is much easier to be misled towards a false positive diagnosis.) 125 smears (7.3 per cent) were classified as dyskaryosis: superficial squamous dyskaryosis—46, intermediate—74, columnar—5.
6. signs of abnormal maturation of the cells.
7. evidence of squamous metaplasia in some columnar cells.
8. degenerated cells, stripped nuclei and cellular debris. Perinuclear halos usually associated with parasitic infection.
9. alterations in vaginal epithelial cells.

The general aspect of the smear and the morphological characteristics of the exfoliated cells make it possible, in most cases, to correctly diagnose acute or chronic cervicitis and endocervicitis by the smear method. Fortunately enough, the above mentioned abnormal cytology is less prominent (less "abnormal") in cervicitis than in malignancy. Nevertheless, the variety of patterns and the diversity of types of the desquamated cells make it difficult to be precisely accurate when describing the exfoliative cytology of cervicitis and endocervicitis.

Discussion

Werner Bickenbach and Hans-Jürgen Soost, Munich, Germany: In our experience the greatest difficulties in judgment are presented by isolated endocervical nuclei, if they are enlarged and their chromatin structure is roughened, and by parabasal cells changed by inflammation. Autolysis in the nuclei with homogenization of their structure nullifies any judgment. Therefore, repeating the smear is obligatory. The appearance of many cocci and mixed bacterial flora, as well as leukocytes and lymphocytes, can only demonstrate a possible inflammatory etiology of the transformation of cells and nuclei. However it does not prove the absence of a malignant process, because the general inflammatory changes may be provoked by a carcinoma.

Concerning the differentiation of inflammatory changes of cells and nuclei in cases of cervicitis and endocervicitis as compared with the actual atypia, we also have the opinion that this is not possible in every case. Cylindrical epithelial cells and squamous epithelial cells from the deeper layers may be changed occasionally by inflammatory influences so much that they cannot be

differentiated from cancer cells in the morphologic picture. The smear should be evaluated if possible as Class III after Papanicolaou and only rarely as Class IV. One can say of suspicious cytological smears with atypical epithelial changes (simply and markedly atypical epithelium, invasive carcinoma as well), which belong to Class IV or V after Papanicolaou, that there one deals not only with inflammatory influences, but with an atypical epithelial change. The question of how far the carcinoma *in situ* can be differentiated cytologically from invasive carcinoma will not be discussed in this paper.

Marcel Gaudefroy, Lille, Nord, France: Dr. Terzano has described perfectly the cytological pattern of cervicitis and endocervicitis. However, I do not quite agree with the statement of Kofler, Kremer and Ulm, who write: "the cytological changes of endocervicitis and cervicitis are identical." It seems to me that there is a great difference between the cytological pattern of ectocervicitis and endocervicitis (when this is isolated). The pattern of ectocervicitis is described extensively by von Haam (the third paragraph of his paper) and Terzano in numbers 1, 2, 3, 4, 5, 6, and 9 of his description.

In pure chronic endocervicitis the cytological changes are less inflammatory and more difficult to diagnose. Stripped nuclei are very common, and their atypical aspect is extreme: very important variations in size, often giant nuclei with several nucleoli, desquamation in strips, similar to endocervical hyperplasia, and very few polymorphonuclear leukocytes. The diagnosis, with malignant immature endocervical cells, is made because the chromatin is always of a smooth appearance. Moreover, one can see giant multinucleated histiocytes which are also found in the endocervical cystic glands of the histological sections. The presence of these giant histiocytes is, in my opinion, a good sign of endocervicitis and a good aid in avoiding a false positive diagnosis for malignancy.

Alexander Meisels, Mexico City, D.F., Mexico: The inflammatory changes described by the main authors are important for several reasons: (a) they may lead the gynecologist to eradicate cervicitis and endocervicitis while the symptoms are still minor, and thereby help to avoid long-lasting irritations, which may be a factor influencing the incidence of carcinoma in this area; (b) they may, in some rare cases, be confused with malignant changes; and (c) they cause great difficulty in the establishment of correct hormonal interpretations.

Héctor Munguia and Esther Franco, Mexico City, D.F., Mexico: We have found cellular alterations similar to those described by the main authors in 90 per cent of the smears studied from cases of cervicitis or endocervicitis. In a few cases there was difficulty in distinguishing benign cellular inflammatory alterations from malignancy (false positives). As Terzano has reported, when this difficulty arises, we found inflammatory changes occurring in deep layers of the epithelium as shown in histopathological studies.

In the remaining 10 per cent no cellular alterations were found, which leads us to believe there may be three factors involved in the production of these alterations: intensity of the inflammatory process, intensity of the response of the epithelium to the inflammatory process, and a combination of these two factors. Therefore, we readily agree with the classification used by von Haam, as an aid in better determining the precise nature of these slightly specific lesions.

P. N. Wahi and Usha Kehar, Agra, India: We have studied the three main papers with interest. Like von Haam our impression has also been that the diagnostic value of exfoliative cytology in benign lesions of the cervix has only been appreciated very recently. It has been our experience that cells with atypical features are encountered where the structure is essentially negative for malignancy, yet these cells cannot be included in the group of normal cells. By and large the most common causes of such benign atypical vaginal cytology are chronic inflammatory lesions and erosions of the cervix. Our comments below are based on a study of 309 cases with erosion of the cervix.

The characteristic cytological features as studied by vaginal and cervical smears consist of the marked exfoliation of basal and parabasal cells, in groups or singly. The cells may look normal or present atypical features with nuclear and cytoplasmic aberrations. The smears contain an increased number and variety of inflammatory cells, including histiocytes, leukocytes and red blood cells. In addition, the smears also vary in their preponderance of ectocervical and endocervical cells. One observes a large number of normal, degenerated superficial squamous cells. The smears also show the presence of an abundant amount of cellular debris.

The changes observed in the basal, parabasal and the endocervical cells are the most significant. Most of these cells are round to oval and may show a slight increase in size. They may occasionally acquire irregular and bizarre forms similar to the tadpole cells, with irregular cytoplasmic processes. These aberrant forms are seen singly or in groups. Some smears show the presence of only anisocytosis while others show both anisocytosis and anisonucleosis. The cellular outline in all these cells is distinct.

The nuclei show an increase in size with a proportional increase in the cytoplasm, thus maintaining the normal nuclear-cytoplasmic ratio. The nuclei almost always retain their normal structure, although at times binucleated and multinucleated cells are seen. The nuclear membrane is intact and regular. The nucleoli may appear distinct, but there is no appreciable increase in their size.

One frequently observes the presence of small cytoplasmic vacuoles. Sometimes polymorphonuclear inclusions are seen. A number of smears show evidence of proliferating and degenerating lesions, as shown by the presence of normal cellular mitosis and the various stages of maturation and degener-

ation. The nuclear chromatin may show slight variations, from evenly dispersed sharply defined granules to dense fragmented particles. Some of these may be seen attached to the inner side of the nuclear membrane forming a complete perinuclear ring. Normal mitotic figures are frequently seen in healing erosions. Cervical erosion thus produces characteristic cytologic changes.

Recognition of these exfoliated cells with atypical features as distinct from benign lesions of the cervix and endocervix is essential in obviating the possibility of major surgery on the basis of a false positive report.

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Closing Remarks

Emmerich von Haam: I agree with Gaudefroy that endocervicitis and ectocervicitis can be differentiated cytologically. However, in order to do this with any degree of accuracy the technique of taking the smears must be highly standardized and very reliable.

Ekkehard Kofler, Hans Kremer and Rudolf Ulm: To Gaudefroy: According to our experience it is almost impossible to distinguish between ectocervicitis and endocervicitis, if the smear is taken with the Ayre spatula.

Guillermo Terzano: The cytologist must be prepared to find abnormal morphological features on cells desquamated from an inflammatory process or a congested mucosa. Describing these features (abnormal cytology, general patterns, limitations, etc.) becomes a difficult task because often it is not easy to express in so many words the knowledge acquired by experience. The interesting remarks given in the discussion make us believe that the subject "Exfoliative Cytology in Cervicitis and Endocervicitis" should be considered as satisfactorily covered.

Colposcopy of Cervicitis and Endocervicitis

JULES ANDRÉ BRET AND FERNAND COUPEZ

Paris, France

CERVICAL inflammations present two very different patterns: primary, on a normal cervical epithelium and secondary, on a cervix with previous benign or suspicious atypia.

I. Isolated Inflammation:

This begins by looking like a colpitis. The ectocervix is uniformly red, congestive, and dotted by multiple, small, dark spots which are invisible to the naked eye. After application of Lugol's solution the colpitis looks like a set of light dots on a brown background.

In the next stage large lymphocytic clusters occur, visible as raspberry-colored dots, which accompany the widespread colpitis. These dots are flat, Lugol negative and, after the Schiller test, look like light areas with faint borders. Up to this time, the mucosa remains unaffected. As the inflammation progresses, necrotic zones appear. They are seen as erosions or ulcerations. These inflammatory erosions and ulcerations may not be identified in a strict sense by colposcopy; however, a local anti-inflammatory treatment causes a rapid cicatrization.

II. Associated Infection:

Ectopy or epidermization is practically always accompanied by infection. For a long time infection was believed responsible for the budding patterns which were unidentifiable. Colposcopy permits, after anti-inflammatory treatment, the recognition of the trophic and inflammatory lesion. Indeed, some benign lesions, such as the ectopy and atypical cicatrization, become unrecognizable when modified by the inflammatory necrosis. When the infection accompanies a dysplastic lesion, it may change the pattern of it enough to make it appear suspicious. In certain cases, the treatment seems to act on the dysplasia itself and causes its regression. Therefore, the part of the infection in the genesis of certain dysplasias may be discussed.

III. Endocervicitis:

A colposcopic diagnosis of endocervical infection may be made only if the ectocervix is healthy, and there is a purulent secretion from the endocervix. The degree of inflammation is not the same for every infectious agent. It is very severe for Trichomonas infestation, where the epithelium is eroded and even disappears in places and bleeds; it is milder with "common" germs, for example, Staphylococci, colibacillus or Proteus, and it is almost absent in cases of pure mycosis with Candida albicans.

ENRIQUE VASQUEZ FERRO

Buenos Aires, Argentina

CERVICITIS is one of the most frequent findings in colposcopy. Due to its polymorphic patterns, its interpretation is difficult even for the well-trained colposcopist. Cervicitis can be produced by many factors, but the local responses have similar characteristics. There are few definite elements on which to base an etiological diagnosis; therefore, a differential diagnosis requires intense training.

Colposcopic examination is supposed to begin by studying the vaginal secretions, before clearing the cervix. Colposcopic patterns vary with localized or diffuse, acute or chronic, cervicitis. For diagnostic purposes it is necessary to recognize the few elements available. Colposcopic diagnosis is sometimes possible and very difficult at other times. In many cases of acute cervicitis where the detection of abnormal epithelia was very difficult, the performance of Schiller's test has permitted our giving a satisfactory diagnosis at a second visit. This was possible by the antiseptic action of the iodine over the acute inflammation.

There are cervical acute inflammations in which an etiological diagnosis can be made, by its typical patterns, on the first visit (e.g., Trichomonas colpitis), but there are many other cases in which we advise treatment of the acute inflammation first in order to be able to perform satisfactory colposcopy at a second opportunity.

The diagnosis of cervical inflammation is not essentially the purpose of colposcopy. Nevertheless, colposcopy is useful for diagnosing some kinds of cervicitis and for aiding in determining the etiology of others. The areas of true erosion can be easily recognized, and the visualization of the free connective tissue is a very important diagnostic element. The complimentary tests can be helpful, and the biopsy is the solution of the difficult cases. Colposcopy is

very useful for determining the origin of menopausal spotting in the cases where atrophic cervicitis and colpitis are present. Also, in cervicitis of pregnancy complicated by cervical bleeding from little capillaries, colposcopy establishes the nature of the spotting.

In chronic cervicitis with a thin epithelium it is possible to recognize the vascular net distribution. This is a very important detail because any abnormalities in quality make a biopsy mandatory. Traumatic lesions (foreign bodies or decubitus) can be recognized, as well as cervical keratinization in prolapse cases which present particular characteristics.

Chronic cervical inflammations can produce changes in the mucosa that can be mistaken for carcinoma in the routine gynecological examination. In condilomata accuminata and papillary verrucous hypertrophy, colposcopy helps in deciding the diagnosis.

Specific inflammations such as tuberculosis, syphilis and diphtheria do not present typical patterns, but some special characteristics aid in the diagnosis. The laboratory will give the definitive word.

Cervical endometriosis can be mistaken for ectopic inflammatory islands, and focal hemorrhages can be found in the premenstrual period.

Colposcopy in endocervicitis can occasionally be helpful. The quality of the mucous tampon is a very important diagnostic guide. Sometimes in cases with patulous external os it is possible to examine the mucosa of the cervical canal.

Colposcopic diagnostic difficulties can be solved in the last instance by making a guided cervical biopsy, but the routine use of this method of diagnosis will save a great number of biopsies.

Discussion

Jean Berger, Basel, Switzerland: The colposcopical picture of cervicitis is characteristic and according to its extension we speak of a colpitis localisata or a colpitis diffusa. This depends on whether or not the squamous epithelium of the entire portio vaginalis uteri or only portions of it display inflammatory changes. The typical pictures are the following:

1. The squamous epithelium of the portio exhibits a reddish discoloration. After painting with 2 per cent acetic acid one finds a dark, finely dotted pattern. These areas, however, are not sharply outlined (as is for instance the "base"). If an ectopy is simultaneously present, one recognizes the grapelike structures only faintly. The tissue is friable and bleeds easily on touch. One finds a brown coloration in most cases with iodine, whereby the formerly dark dots now appear white, the reason probably being the loss of glycogen perivascularly. This picture is characteristic.

2. There are also other forms of colpitis-cervicitis. One finds small whitish areas diffusely distributed over the cervix. At first glance one thinks it a leukoplakia. These small whitish patches are already found without iodine application, but are more pronounced after painting with iodine. Very frequently this type of colpitis is found in cases of Trichomonas infestation.

3. An ectopy or a beginning transformation zone may display inflammatory changes, without showing the characteristics described above. In such cases one finds vulnerable tissue, slight bleedings and characteristic proliferations of the cylindrical epithelium or of the squamous epithelium. The tissue stains partly positive and partly negative with Schiller's test. These types of colposcopic pictures are often mistaken for abnormal or atypical transformation zones, since they are very difficult to evaluate. In any case, we recommend a biopsy because a carcinomatous growth is too easily overlooked.

A characteristic colposcopic picture for endocervicitis is not well defined, the reason being that even under maximal spreading of the external os the endocervix cannot be fully viewed.

Herbert Janisch, Vienna, Austria: It is most gratifying that the vast field of colposcopical differential diagnosis of the various forms of inflammation is mentioned. A complete evaluation of this cannot be accomplished in this short discussion. For differential diagnostic purposes the fact that there are practically no variances in the focal place of the colposcopical picture, disregarding some specific inflammations, seems important to us. In ul-

cerations a sound test under the guidance of the colposcope is often very helpful. Also, an observation of the type of discharge is important. Conclusions as to the etiology, however, can only be done with caution. Bacteriological examinations seem indispensable to us. In cases of specific inflammations, a biopsy and histological diagnosis is necessary.

Warren R. Lang, Philadelphia, Pennsylvania, U.S.A.: I agree in the main with Bret and Coupez. With portio inflammation there is not extensive loss of epithelium but rather multiple minute areas of true erosion; these take the iodine stain poorly. That regression of colposcopically atypical areas can occur after treatment for inflammatory processes is true; it can also occur spontaneously.[1]

The colposcopic technic is of value in studying leukorrheas as Ferro suggests[2] and especially with regard to Trichomonal inflammations.[3] I would be loathe to base a final diagnosis of postmenopausal bleeding on colposcopic examination alone in cases of atrophic cervicitis and vaginitis; examination under anesthesia and diagnostic curettage are usually required in such cases.

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Closing Remarks

Enrique Vasquez Ferro: The difference between localized cervicitis and diffuse cervicitis is well known. It is not always possible to picture a definite colposcopical pattern, because rather frequently there are associated and/or concomitant processes. In sections, inflammatory reactions in the connective tissue are often observed in the common processes of reparation. We agree with Janisch that for the differential diagnosis of some non-specific cervicites, colposcopy may help, but only to some extent. Also, it may help to locate some postmenopausal bleeding in atrophic cervicitis and colpitis, as Lang states.

Colpomicroscopy of Cervicitis and Endocervicitis

VIKTOR GRÜNBERGER

Vienna, Austria

THE colpomicroscopical findings in cervicitis and endocervicitis are not very impressive, since the view is impaired by the mucus which flows from the external os. Usually only the toluidine blue stained mucus plug can be visualized at the site of the external os, in which various cellular elements may be recognized. In cases of the true, but very rare, inflammatory cervicitis, one finds large numbers of leukocytes, lymphocytes and cellular debris. Also, nonviable cylindrical cells from the cervical canal and the mucus glands are frequently encountered. In women in the childbearing age, the quality of the cervical mucus, of course, depends upon the phase of the menstrual cycle. At the time of ovulation the mucus becomes clear and contains only a few cellular elements and at the time of ovulation the external os gapes (is half open), so that one can sometimes even see changes at the transitional zone between the external os and the endocervical canal. An examination of the endocervical canal itself with the aid of the colpomicroscope is not possible.

Discussion

Marcel Dargent and Pierre Haour, Lyon, Rhône, France: The observation of infected epithelium is much easier after cleaning the cervix thoroughly with water before staining it with toluidine blue: it not only cleans the lesion of mucus and autolysed elements, but it also gives clearer details of the cells.

We think that it is sometimes impossible to make a correct observation of the epithelium around the cervix, when mucus secretion exists at the cervical os.

Wolfgang Walz, Heidenheim, a.d. Brenz, Germany: In addition to the findings described by Grünberger, one may sometimes succeed in distinguishing various degrees of inflammation. In the acute stage one finds merely inflammatory cells, cellular debris and fibrin on the surface. In the case of a chronic inflammation, one finds a moderately infiltrated connective tissue on the surface in which remnants of mostly nonmucus producing columnar epithelium may be present. As Grünberger states, endocervicitis can only be recognized indirectly by changes in the cervical mucus. Thus in this case, only a suggestive diagnosis can be made.

Clinical Viewpoint on the Management of Cervicitis and Endocervicitis

WARREN R. LANG

Philadelphia, Pennsylvania, U.S.A.

ALTHOUGH the terms cervicitis and endocervicitis are frequently used interchangeably, cervicitis literally means inflammation of the entire cervix; endocervicitis, inflammation solely of the endocervix. Should the inflammation be due to an infectious agent, then one must distinguish cervical flora from cervical infection. It would seem that the cervix is truly infected if (a) there is a pure culture of a microorganism which may be found in the normal flora or (b) there is some other microorganism of accepted and undoubted pathogenicity present.

But, the terms cervicitis and endocervicitis signify various concepts to different people. Probably the commonest usage of the terms relates to the presence of a benign cervical "erosion," usually with laceration, eversion and cystic change as seen in multipara. These manifestations are often accompanied by mucoid or mucopurent cervical secretion. Although a so-called benign "erosion" looks inflamed to the naked eye, it is now fairly generally accepted that the basic abnormality, if it is truly abnormal, is the presence of columnar epithelium on the portio.[2] Since columnar epithelium is less opaque than squamous epithelium, the vascular stroma shines through, thus accounting for the red color. Such a "cervicitis" is quite common in the childbearing era and diminishes greatly in incidence in the postmenopausal era.[4] During pregnancy the cervix demonstrates hyperplastic changes, especially of the columnar epithelium, and this causes the area to appear red and angry.[3] The presence of columnar epithelium in a portio biopsy of a pregnant or nonpregnant cervix may be interpreted by the pathologist as signifying the anatomical endocervix, but this, of course, is not necessarily so.

A discussion of management must of necessity be related to whichever of the above concepts of cervicitis-endocervicitis is adhered to. If one is attempting therapy of an actual infection with potentialities of

spread to the uterine cavity and above, systemically administered antibiotics may be advisable (e.g. for gonococcal infection). If a local cervicovaginitis is present, then local methods of treatment are required but not always completely successful. So-called nonspecific cervicovaginitis yields usually to treatment with local antibacterial or antibiotic agents. Candidal infection is managed by higher fatty acids or antifungal antibiotics. Trichomonal infections are managed with antitrichomonal agents containing such compounds as pentavalent arsenicals, iodine compounds, furoxones and a host of others. Purely local cervicovaginitis is also treated by various cleansing douches. Literally hundreds of techniques and preparations have been tried.

The objective in treating benign cervical "erosion" with its associated changes is to have the portio ultimately covered with squamous epithelium. Purely medical means such as douches, creams or jellies are unable to do this, although they may serve to diminish concomitant inflammatory changes. Local caustic agents, such as a silver nitrate stick, may destroy the epithelium sufficiently to allow squamous epithelium to grow over. More efficient, however, is direct cauterization with an electrocautery. Many technics have been devised to cone the cervix (cone, ring, circular, annular biopsy) so that a sufficient rim of portio and endocervix are removed. Our personal preference is to perform this using a scalpel and marking any grossly suspicious area with a pin or even excising it separately.[5] Such a biopsy has not only diagnostic value for the pathologist, but therapeutic value as well, since often the cervix resembles an intact, nulliparous cervix when it has healed. An amputation of the cervix (trachelectomy, cervicectomy) may be desirable in a greatly hypertrophied cervix. If concomitant corporeal pathology is present, then a complete hysterectomy by the abdominal or vaginal route is indicated.

The question might well be posed as to the need for treating such a cervix if by cytology, possibly by colposcopy, and ultimately by biopsy, the cervix shows benign changes. Probably the most potent argument for this is the work of Cashman[1] and of others showing that if such a cervix is treated, invasive carcinoma is less apt to develop later in life.

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Discussion

Jean Berger, Basel, Switzerland: The clinical approach in the therapy of cervicitis and endocervicitis depends upon the changes observed.

First of all a bacteriological evaluation should always be done in addition to the cytological examination. For all practical purposes the preparation of smears from the posterior fornix, the ectocervical surface and the endocervix will suffice. The specimens should then be stained with methylene blue or according to the Gram technic. In selected cases a resistency determination might be worthwhile in order to institute proper therapy.

If one finds the typical signs of cervicitis on the cervical surface, the condition first should be treated. The following therapeutic methods are available today:

1. Chemical substances, such as silver nitrate, Negatol and others which enhance the regeneration of the squamous epithelium.

2. Electrocoagulation whereby the inflamed epithelium is cauterized and the normal squamous epithelium is stimulated to regenerate.

3. Surgical removal of the inflamed tissue.

I believe that the first two methods can be especially helpful, provided that they are used

intelligently and preceded by the proper diagnostic evaluation, such as colposcopy, cytology, biopsy and curettage, if necessary.

It is of importance to note whether or not inflammatory changes, easily bleeding ectopies and erosions still exist on the surface of the cervix since these conditions favor the discharge and the persistence of the cervicitis.

Frantisek Luksch, Prague, Czechoslovakia: Referring to the therapy of endocervicitis as roughly outlined by Lang in his contribution, I would like to add that in our institution we have achieved very favorable results with electro-coagulation in inflammatory cervical conditions, excluding the so-called "benign erosions." The ball-shaped electrode is pulled through the cervical canal once or several times and the epithelium coagulated. The healing processes are probably of the same kind as the ones observed in healing after coagulation of benign cervical lesions. In both cases we have never observed unfavorable results, such as strictures, etc. A combination of this therapy and the antibiotic or bacteriocidic substances, as introduced by Lang, seems to us the therapy of choice at the present time.

Marco Marcov, Stara Zagora, Bulgaria: Dr. Lang describes almost every method for the treatment of cervicitis and endocervicitis. More and more drugs are coming into use and are recommended. In spite of this, recurrences are often observed.

In my own practice I use mainly electro-coagulation and do so with success.

An undesired complication is the bleeding, sometimes of long duration. In one patient the hemorrhage was so severe that she had to be hospitalized and given two blood transfusions.

The true localized inflammations of the cervix and the vagina may also be quickly treated with 8 per cent silver nitrate.

Closing Remarks

Warren R. Lang: There is little to add to the comments of the discussers.

I would like to emphasize, however, the statement of Berger that microbiologic studies are valuable at times with sensitivity determinations. In some cases these enable a specific diagnosis to be made and specific therapy to be instituted. This is often overlooked but should be done, especially in refractory cases of vaginocervicitis. The necessity for biopsy in questionable cases of cervicitis-endocervicitis is again stressed.

Interrelationship: Cervicitis-Endocervicitis and Cervical Carcinoma

JAN HEROLD

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IN a preceding paper (see "Letters to the Editors" Vol. III, No. 1 entitled "Abnormal Neurological History of Patients with Early Carcinoma"), I reported on a clinical observation, that the appearance of gynecological cancer, and that of other organs as well, is regularly preceded by signs of slight, repeated, chronic encephalitis, probably of virus origin, which might be considered one of the main causes of malignant growths. I also mentioned that virus encephalitis, most probably basal, causes disturbance of the central trophic regulation, which, according to its localization and intensity, evokes a corresponding trophic disturbance of the peripheral tissue area. As a culmination of these changes, a malignant growth then develops.

According to this conception I shall now endeavor to explain biochemically the mechanism of the pathogenesis of cervical carcinoma and prove my investigations with some photomicrographs.

The important characteristic of viruses —fundamentally, simple nucleoproteids—is their obligatory biotropism. It is due to

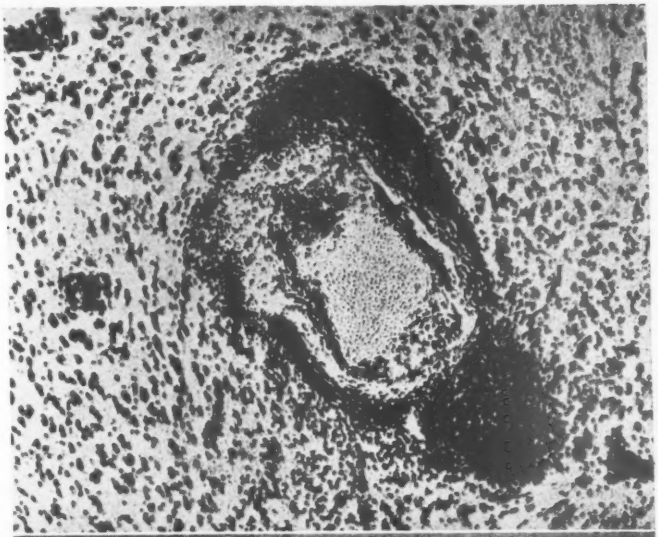


FIG. 1. Acute basal encephalitis in a case of periarteriitis nodosa.[5]

FIG. 2. Section of basal ganglia of the brain of a woman, dead of cervical carcinoma. Hematoxylin-eosin.

the fact that the virus generally lacks its own important enzyme groups essential to its metabolism and multiplication. These enzymes must be provided by the host. Viruses, penetrating into the organism or already present, reach the basal ganglia of the brain by means of the circulatory system where they themselves, being of protein nature, attack cellular nucleoproteids, in order to utilize their enzymes (especially respiratory enzymes containing ferrum, which are found in great quantities in basal ganglia and cause a brownish coloring of the substantia nigra and nucleus ruber). The reaction with enzymes is obviously brought about by the attraction of

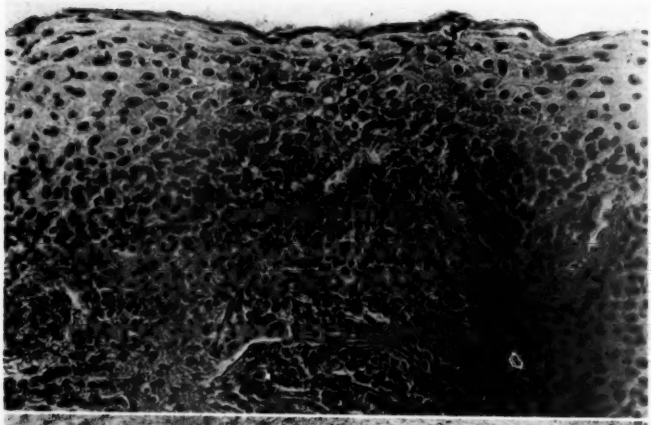


FIG. 3. Perivascular cervicitis and atypical epithelium of cervix of a woman with positive cytological smear. Hematoxylin-eosin.

FIG. 4. Fine perivascular infiltrates in basal ganglia of boy dead of Hodgkin's disease. Hematoxylin-eosin.

electrical charges. The normal protein molecule has rather a large surface potential and its desmoenzymes are closely attached to it. Physiological aging of living substance, innate causes, frequent virus attacks, and probably also mental shocks lower the electrical potential of the protein molecule. The binding of the enzymes becomes loosened and these may then more easily cohere to present viruses, i.e., deviation of enzymes. The damaged molecule is condemned to destruction and to its own detriment offers building material to the virus, which then multiplies.

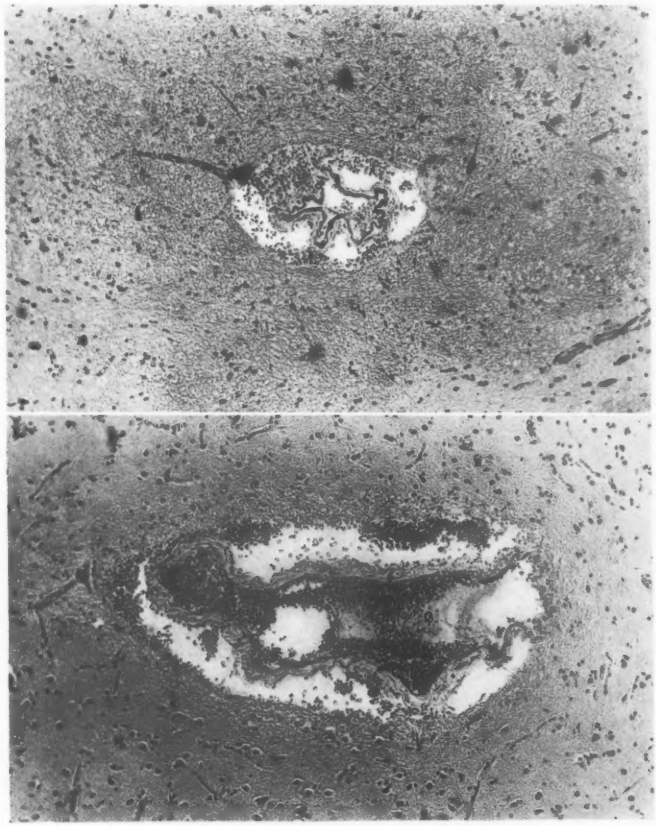
The analogical process takes place simultaneously, practically telegraphically, at the corresponding peripheral area: the fall in the electrical potential in central cells and tissues is signalized by the centrifugal nerve passage to the peripheral cells and tissues where enzymes, freed from protein bindings, become the prey of the same species of virus. The increase of viruses in the center and periphery as well creates an inflammatory process, which primarily and more frequently attacks the vasomotors of the smaller arteries and thus forms perivascular infiltrates composed chiefly of lymphocytes and plasmocytes.

This picture of basal encephalitis appears to be a common source of numerous diseases of as yet unknown etiology (e.g.,

collagenoses — periarteriitis nodosa — see Fig. 1).

In persons who died due to a malignant growth we find, because of the prolongation of the disease, the above mentioned inflammatory focuses in stages of regression to complete recovery: Figure 2 shows a section of basal ganglia in a woman of 53, diagnosis, cervical carcinoma. Through the destruction of the adventitia vacant places or fine reticulum develop, containing in some parts the still visible remains of an inflammatory infiltrate. Vacant places can arise artificially through the tearing off in the working up of the material; however, the cause of the tearing off is the pathological process which had taken place prior to histological examination. In the environs of afflicted vessels we sometimes observe a decline or even a complete disappearance of neurons, and neuroglia exuberance.

Let us now return to the periphery: there the perivascular infiltrates cause damage of the vessel walls and blockage of the oxydases. This results in the hypoxia of the tissue which is nutritionally dependent on the damaged vessels, and leads consequently, to the atrophy and finally to disintegration of tissue elements. The squa-



FIGS. 5, 6. Destruction of adventitia and extravasates of erythrocytes in basal ganglia of person mentioned in 4. above. Hematoxylin-eosin.

mo-columnar junction of the uterine cervix is just such a peripheral area, possessed of a lively regenerative and metabolic activity of the epithelium. Therefore the respiratory enzymes here are thickly crowded, their offer to the metabolism of viruses is more abundant and thus the readiness of the epithelial proteins and of the cells themselves to disintegration is greater. In this manner the well-known predilection of the origin of cancer in women on the uterine cervix becomes explicable. Figure 3 shows a perivascular cervicitis and an atypical epithelium as the initial cancerization of the uterine cervix in a woman with a positive cytological smear.

The atypical epithelium of the vaginal portio is a further link in the pathogenetical chain of carcinoma of the uterine cervix. Virus cervicitis is the cause of trophic disturbance of the corresponding area of the epithelial tissue. Normal cellular proteins of the epithelium disintegrate as a sequence of hypoxia; freed redoxases, especially plentiful in cellular nuclei, are utilized by similar virus proteins. These then increase intensively in number to the detriment of nuclear proteins, whose composition they imitate to an exaggerated extent, creating something like caricatures of normal nuclei. Thus enlarged and deformed cellular nuclei, the basis of cytodiagnostics of tumors, arise—the result of intranuclear virus parasitism.

The reversibility of the process depends on the actual state of the central trophic regulation. Its absolute disappearance evokes an autonomous tumorous growth. The principle of cancerogenous mutation would thus be a complete inversion of normal protein metabolism into the metabolism of virus proteins, which form the main component of tumorous substance. It would be the culmination of the process, which at its lowest stage appears as physiological aging of man.

Vessel changes of basal ganglia, visible in Figure 2, are often considered by pathologists as malatic foci of arteriosclerotic origin. I also found similar vasculopathies, however, in basal ganglia of the brain of a 15-year-old boy, dead of malignant lymphogranuloma (Hodgkins's disease). Macroscopically these basal ganglia were normal; the microscope showed fine perivascular infiltrates surrounding the smaller vessels (Fig. 4) and in several parts a grossly obvious destruction of the vessel adventitia (Fig. 5, 6).

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FRANCISCO NOGALES

Madrid, Spain

THERE are many clinical reports about the carcinogenic role of the inflammatory diseases of the cervix.[2-9]

Hinselmann states that he has never seen one carcinoma arising from a cervix with a pin-hole sized external os and intact mucosa.

Hamperl and co-workers show that the

origin of the cervical carcinoma is in the prolapsed columnar epithelium in contact with the vaginal environment.[1]

Nevertheless, gynecological pathologists, so inclined to accept the hormonal role in the genesis of cervical carcinoma, are cautious and distrustful about the carcinogenic role of chronic inflammation.

Based on study of 170 cases of tuberculosis of the cervix, up to the present moment we have described[6, 7] the alterations of the stratified and cylindrical epithelium due to chronic irritative stimulus produced by the tubercles of the stroma. We have seen cylindrical epithelium undergoing important functional alterations and morphological changes, namely vacuolization, pyknosis and regressive nuclear atypias. On the other hand, we have also seen the metaplastic transformation of the cylindrical epithelium into squamous epithelium, cellular and nuclear atypias, hyperchromatism, increase in the proliferative activity of the epithelium, and, finally, as if it were an experiment, we have been able to follow in one case the malignant transformation of the cylindrical epithelium producing an epidermoid carcinoma of the cervix. In the same biopsy, partially taken by punch and partially taken by endocervical curettage, one can see the phases of transformation of a normal glandular epithelium into a metaplastic epidermoid carcinoma. In one single gland, one can see part of the unchanged epithelium and part in metaplastic proliferation which fills the lumen of the gland (Fig. 1-5).

We firmly believe that inflammatory diseases of the cervix usually result from delivery which, due to the anatomical alteration, allows the penetration of septic secretions that have physical and chemical properties harmless to the squamous epithelium but not to the ectopic endocervical epithelium. It is well known that epidermoid metaplasia of the cylindrical epithelium is due to chronic inflammation of the cervix

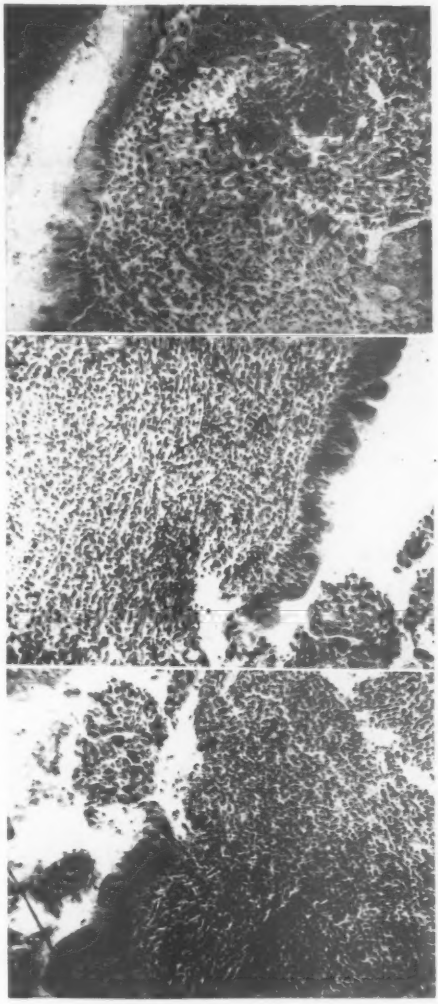

FIG. 1 (Top). Cervical tuberculosis. 35 years old. Sterility. Metrorrhaghia since 8 months of age. There is a papillary, friable, partially ulcerated lesion of the cervix. Microscopically there are several confluent tubercles with some giant cells and lymphocytic infiltration. The glandular epithelium near the lesion shows mucous-producing activity. This is a frequent finding in glands near tubercles. FIG. 2 (Center). In another microscopic field one can observe the epithelium with the same characteristics, but in the lower portion it has undergone malignant metaplastic transformation. FIG. 3 (Bottom). The same histologic field—in order to show the continuity of the tumoral epithelium with the mature cylindrical epithelium.

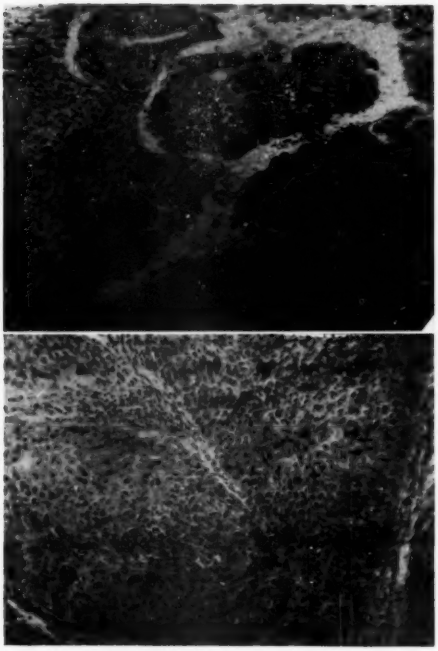

FIG. 4 (Top). In another microscopic field the "partial" transformation of the clear epithelium of a cervical gland into a hyperchromatic and atypical epithelium can be observed. FIG. 5 (Bottom). In another microscopic field of the same biopsy a solid mature epidermoid carcinoma can be seen.

in ectropion, laceration or during pregnancy.

In our opinion, the carcinogenic agent which overlaps genetic factors is, with all probability, in the cervix, the chronic inflammation of any origin, although it may be that vaginal parasites (Trichomonas) have a more selective action.

In our opinion, the studies relating hormonal stimulation as a carcinogenic factor are hardly convincing. Our review of operative specimens in cervical carcinoma agree with Martzloff's report that the endometrium and the ovary do not undergo anomalies.

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Discussion

Hans F. Bettinger, Melbourne, Australia: Many of my clinical colleagues are absolutely convinced that one of the most important factors in the causation of carcinoma of the cervix is chronic inflammation. On the other hand, among the innumerable cervices that we have examined from uteri removed for benign conditions, I don't think there has been one that did not contain some inflammatory infiltrations. They are usually not very extensive, but they are always present, and this is why I am doubtful of whether or not inflammation plays a decisive role in the origin of cancer.

Warren R. Lang, Philadelphia, Pennsylvania, U.S.A.: The term cervicitis, at least to the United States gynecologist, signifies a so-called benign "erosion," a true inflammation of the cervix, or both. Strictly speaking, it should indicate a purely inflammatory process. The relation of cervicitis in this restricted meaning to cervical carcinoma is in the over-all problem of development of carcinoma from inflammatory processes in general.

The concept of cervical carcinoma arising from encephalitis, or even from viral cervicitis, is frankly quite new to me. Before it is accepted, certainly a great deal more investigative work is required. What are the normal viruses of the cervix? How do these differ from those in cancer? Are the encephalitic findings described by Herold found in normal women without cancer? Controls and statistical data are necessary. There have been some stirrings in the field of vaginal virology but, to my knowledge, nothing in the way of an overall appraisal has yet been published.

Nogales states that the studies relating hormonal stimulation to subsequent cervical cancer are not

convincing. To this we would almost all agree. Probably the organism most frequently implicated in infectious processes is the vaginal trichomonad with the paper of Bechtold and Reicher[1] most frequently quoted. In our institution (Jefferson Medical College Hospital, Philadelphia, Pennsylvania) there has been one case of concomitant cervical tuberculosis with cancer in approximately 990 consecutive ward cases of carcinoma of the cervix since 1921.

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Closing Remarks

Jan Herold: The often found cervicitis in benign cases, mentioned by Bettinger, is proof of how rarely the condition of perfectly normal health appears. It is quite conceivable that the majority of cervical inflammations does not necessarily develop into carcinoma. The decisive factor is, according to my conception, the actual state of the central trophic regulatory system localized in the basal ganglia of the brain. The assimilation and dissimilation of the healthy peripherai tissue is regulated by normal, functionally efficient ganglion cells of the basal ganglia. Such cells are of polygonal shape with nerval prominences, rounded or oval nuclei with a well-defined nuclear membrane, a fine chromatin structure and one single, obviously dark nucleolus (Fig. 7). Their protein molecules have sufficient surface electric charges to attract enzymes essential for metabolic activity and function of cells. The disturbance of central trophic regulatory system arises due to the decrease of electric charges in the proteins of ganglion cells. The released enzymes serve to help the multiplication of viruses and thus viral basal encephalitis originates. This pathological process can be seen, according to my opinion, in morphological and tinctorial changes of some ganglion cells: in addition to morphological changes of the cytoplasm we also observe a definite polymorphism and hyperchromasia of nuclei, most likely in the sense of dyskarypsis. Figure 7 shows the histological picture of basal ganglia of a 53-year-old woman who died of cervical cancer (the same case as shown in Fig. 2 of my original paper).

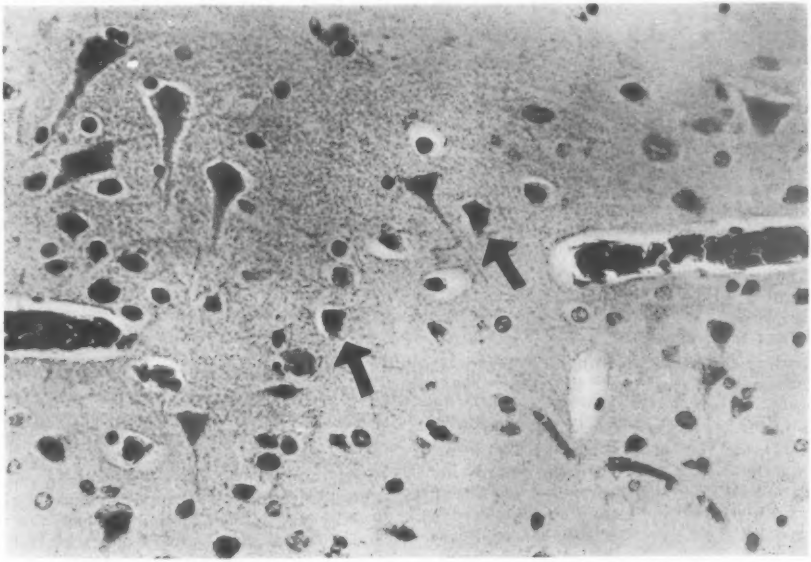

FIG. 7. Histological picture of basal ganglia of the same patient as shown in Figure 2. Beside several normal neurons two elements with nuclear changes /↑↑/.

I am convinced that the disturbance of central trophic regulation is the primary cause of numerous pathological processes in the body. The decrease of electric charges in the center is signaled to the corresponding area of the periphery, where an imitation of the central changes arises, however, to a comparatively larger extent. The center is connected to the periphery by the vegetative nervous system, most often by the perivascular sympathetic plexuses. Therefore, peripheral viral inflammations first afflict adventitia of the vessel, creating perivascular infiltrates of lymphocytes and plasmocytes. The proper tissue elements, dependent for nutrition on the afflicted vessels, then react pathologically either by the multiplication of viruses (degenerative changes, hypertrophy, hyperplasia, tumors), or by hypotrophy to atrophy, in case of the dissolution of central trophic neurons. Also microbial infections, e.g. tuberculosis, typhoid fever, phlegmona, sepsis, etc., may successfully develop only in a terrain the central trophic regulation of which is disturbed. The decisive factor in this case is also probably the utilization of the enzymes of the host, which only then makes possible the clinical manifestation of the infectious disease.

Lang correctly remarks that the general problem, reaching far beyond the close relation between the inflammation of the uterine cervix and carcinoma of this organ, is in question. Due to the fact that my findings are based on numerous clinical observations, I consider it important to mention on this occasion some questions, even if at times they are not closely linked with gynecological oncology.

To answer Lang as to whether or not the encephalitic findings are found in normal women without cancer, the answer is yes; in persons without cancer, as seen in Figure 1 in periarteriitis nodosa. I am certain that in collaboration with a neuropathologist, one could find signs of basal encephalitis in the majority of deceased patients. Personally, as a gynecologist, I have not as yet had the opportunity of gaining a most definitely rare histological picture of the basal ganglia of a normal healthy individual (e.g. a perfectly healthy child accidentally killed).

In answer to Lang's question: "What are the normal viruses of the cervix? How do these differ from those in cancer?"

According to one of the two main theories on the nature of viruses some viruses are not of external origin at all, but are derived from the cell proteins of the organism.[1] This theory signifies that the very same virus may appear in a dual role: once as a "normal" virus, derived from tissue proteins, its character being of intricate polypeptide chains, an ubiquitous symbiont of each metabolizing tissue. On the other hand, when transferred to a heterogenous body, it behaves as a pronounced parasite, reacting antigenously, due to its specifically different molecular structure.

Normal viruses are the natural intermediate products in assimilation and dissimilation of proteins. They do not damage the maternal organism, because by their normal multiplication and synthesis, functionally efficient tissue proteins again originate. In this way, in cases of tissue defects, they function restitutionally and reparatively. Normal viruses, due to their high activity, also behave defensively against infections and other antigens. Meanwhile, the basal ganglia of the brain act as the center of coordination for all regenerative and defensive processes in the body. Thus it is possible, considering the intensive activity of normal viruses, to explain the origin of all defensive, morphologically different elements of the active mesenchyme (RES), their capability of phagocytosis and production of antibodies. It is quite obvious that the following are of viral origin: plasma granules of mastocytes, eosinophilic Russel bodies and binucleation of plasma cells, hypertrophy of plasma and vesicular enlargement of the nuclei of histiocytes, multinucleation of giant cells and pleiomorphism of reticulum elements. In creating the granulation tissue, besides fibrin and the above mentioned cells of the active mesenchyme, fibroblasts also participate, i.e., virally mobilized fixed connective elements, and furthermore similarly activated vessel endothelia, proliferating into capillary loops.

The acquired antibodies are specific globulins, produced by active mesenchym cells.[2] We can imagine that the infectious agent (antigen) penetrates through to the trophic nerve center for the regulation of RES activity. During the fight for enzymes between the normal protein molecules and antigens (heterogenous virus, bacteria, toxin), in ganglia cells certain prototypes of antibodies originate, whose creation is signaled by way of the nerve passage to the active mesenchyme cells. Thus a great number of specific antibodies arise in the RES, without having been in any contact whatsoever with the antigen. This conception is mostly similar to Burnet's modern theory. This theory, however, assumes the formation of a new enzyme, modified by antigen, and does not take into consideration that the creation of antibodies is centrally controlled.

Furthermore, it is well known that antibodies appear under two distinct molecular weights: the one type equals 160,000 to 175,000, the other approximately one million. Before the origin of normal antibodies a certain kind of imperfect, small molecular pre-stage originates in the tissues, soon replaced by antibodies of higher molecular weight.[2] It is highly probable that the original antibodies of a small molecule are the products of normal cell viruses, the organism being capable of quickly creating the required amount. Their high activity and prompt origin do not permit the patient to succumb to the first attack of the infection during the period, before the definite antibodies of large molecules and globulin composition are produced.

In case of a disturbance of the vegetative regulation in the center for RES, i.e., by an innate or acquired decrease of electric charges of the normal proteins and their normal components as well, the corresponding enzymes (redoxases) become released and, owing to their deviation, heterogenous viruses multiply. In this way, in contact with the antigen, prototypes of pathological, incomplete antibodies arise centrally, instead of full antibodies. The peripheral answer of the active mesenchyme cells leads most often to a permanent production of various amounts of these heterogenous viruses, which are distributed throughout the circulation or localized in tissues. Thus, we can explain a lot of obscurities in the pathogenesis of allergies, eczemas and immunotoxic diseases.

Also fetal erythroblastosis originates analogously: at the end of the pregnancy single fetal erythrocytes, or their RH antigens respectively, evoke in the pathologically reacting mother the production of incomplete antibodies, arising practically explosively in the RES. These substances penetrate in an enormous amount through the placenta and, due to their high activity, damage the erythrocytes of the fetus, obtaining oxydases and the active oxygen from them. In the multiplication of viruses, atoms of hydrogen become released from each molecule of the viral protein (dehydrogenation), always finally binding the oxygen to a water molecule. The quantity of produced water can thus indicate the intensity of the viral metabolism. We observe this general appearance not only in the fetal erythroblastosis (hydrops fetalis), but also in various inflammations and tumors (spongiosis of the epidermis in eczema, balloon and reticular degeneration of cells in herpes, the content of ovarian cysts, ascites in carcinoma peritonei, aqueous discharge in cervical carcinoma, perimalleolar edema in patients suffering from cancer, etc.).

Also naevi pigmentosi are most probably of viral origin. They develop singly on the skin of many persons, but are often thickly crowded on the skin of the face, back of the hand, forearm or perigenital area in patients with malignant growths. Histologically they are composed of nevus cells, derived from melanocytes ("clear cells"). Melanocytes are now mostly considered as elements of nervous origin, traveling up to the skin in the embryonal period.[3] The connection of naevi pigmentosi with the central nervous system is especially striking in this case. I am convinced that, due to the disturbance of the central trophic regulation, the deviation of tyrosinase and other enzymes (polyphenoloxydases) arises in melanocytes of an exactly limited place of the epidermis. These enzymes, which make the successive change of tyrosin into melanin possible, are utilized by heterogenous viruses, containing tyrosin as well. In this way melanocytes change into nevus cells showing at times two amitoticly divided nuclei, several nucleoli and melanin granules in the plasm. By the limited proliferation of nevus cells and the hyperproduction of melanin, various clinical pictures of these skin malformations then originate

Finally, I should like to add a few words on the problem concerning the cornification of the vaginal epithelium.[4]

Cystin and cystein respectively, as the main components of keratin, are also the regular constituent of viral polypeptide chains, where they function in oxydo-reductive processes as a hydrogen carrier, perhaps bound to the tripeptide glutathione. In the proteosynthesis of healthy vaginal epithelia the above mentioned normal virus participates, its assimilation is nervously regulated, can be influenced by administration of estrogens, and develops into the keratin-hyalin or pre-keratin stage respectively, with a greater content of sulfhydryl groups. In pathological conditions, when the central trophic regulation is disturbed, heterogenous viruses actively multiply, utilizing the deviated enzymes of normal proteins. Their proliferation is of an autonomous character and by dehydrogenation of cystein, numerous disulfide groups are produced as well as various quantities of true keratin, which, finally, forms the main pathological constituent of leukoplakia and carcinomatous horn pearls. Thus two very similar processes are in question: one of them is of physiological, the other of pathological nature. Both processes certainly often go over into each other or are closely related. I find that, in this way, one can explain some divergent views or controversies of discussants considering the problem of the cornification of normal vaginal epithelium. I assume that even in the use of standard histochemical technics the results will probably not be uniform.

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Closing Remarks

Francisco Nogales: Lang cites a case of cancer and tuberculosis among 990 cases of cervical carcinomas. We do not give tuberculosis a special carcinogenic meaning. However, we choose the more expressive case which we have in our files and photographed for demonstration in our paper.

I agree with Bettinger that it is rare, in the systematic sections of cervices arising from operative specimens of biopsies to find inflammatory infiltration of the stroma. We also find this to be true in metaplasias of the ectopic cylindric epithelium. We have not seen one case of carcinoma *in situ* that does not have an inflammatory stroma.

Histomorphology of Ectopy, Ectropion and Epidermization

JEAN A. DE BRUX, HENRIETTE WENNER-MANGEN

Paris, France

AN ECTROPION is the polypoid eversion of the fringes of the endocervical mucosa, protruding at the external cervical orifice.

Cut longitudinally in its long axis, it is seen to have a short, wide pedicle which branches out into arborescent fringes: the pedicle is simply the elongation of the submucous connective-tissue. The latter appears loose-textured, edematous, and contains a few fibrocytes. The vascular pedicle gives rise to a multitude of dilated capillaries with edema. Finally, there are noted a few hypersecreting mucous glands opening by a canal onto the surface of the ectropion.

In the sub-epithelial zone there are often found patches of adenomatous mucous glands opening widely at the exterior. The border epithelium is columnar and often very secretory. However, it may become abraded, or altered by epidermoid metaplasia. Finally—and most frequently—the ectropion becomes inflamed, with infiltration of lymphocytes, plasmocytes and polynuclears, sometimes to such an extent that the ectropion becomes only an inflammatory granuloma, having lost all recognizable morphology of an ectropion.

The ectopy is formed by a zone of columnar epithelium in the midst of squamous cell epithelium.

Virchow, who had observed such ectopies on the cervices of newborn female infants, advanced the theory of an embryonic origin. Such anomalies may in fact be found in pre-adolescent girls and are manifested by a banal leukorrhea, often difficult to cure; they may likewise be seen with the colposcope.

In the adult woman, however, they are acquired and their origin may be the result of (a) traumatism or an inflammation having ulcerated the squamous cell epithelium and removed a fragment of the chorion, thus bringing into contact with the vagina the fundus of a mucous gland, the inflammation and the infection hindering rapid cicatrization or (b) in most cases the ectopy is simply the remainder of an ectropion whose edges are in process of cicatrization by metaplastic epidermization.

Epidermization: The epidermization by squamous cell metaplasia of the columnar cells is one of the evidences disproving the theory of the immutability of cells. Its mechanism is the following: At the foot of each columnar cell there exists a "reserve" element, which, when the cell dies and exfoliates, grows and replaces it morphologically and functionally. In certain abnormal conditions of life or in certain infections and intoxications (whooping-cough, effect of smoking on the bronchial epithelium), these cellular elements do not evolve normally. We then see:

(a) a multiplication and hyperplasia of these reserve elements, which become contiguous; (b) their multiplication is such that they pile up; (c) differentiation occurs, not as columnar but as squamous cell epithelium; (d) stratification occurs and later maturation.

The remaining active columnar cells are thus lifted by a sort of squamous cell base. As shown by the muci-carmin stain, these remaining columnar cells have still conserved their secreting character; several weeks later, they exfoliate, after undergoing certain modifications. While these columnar cells, covering the metaplasia, sometimes remain perfectly normal, most often they, too, present an altered aspect; they become voluminous, hyperplastic, with large nuclei and chromatin of increased density, prominent nucleoli and slightly thickened nuclear membranes. The cytoplasm becomes more cyanophilic and sometimes vacuolized. The limit of this epidermization coincides with that of the ectropion or of the ectopy and is distinctly visible in the Schiller test. On the biopsy, this newly-formed, squamous cell epithelium sometimes shows an exophytic, wavy aspect, and the contours of the peripheral endocervical fringes and papillae are easily seen. More often, this wavy aspect appears at the lower part of the epithelium. The glands situated below the columnar epithelium are penetrated by the same epidermoid metaplasia. Here and there persist a few glandular secretory canals, forming a sort of small "chimney" in the midst of this epidermoid epithelium. (This aspect is well known in colposcopy.)

The newly formed epithelium thus shows digitations of varying thickness, separated by bands of connective tissue. The squamous cell epithelium thus constituted, sometimes hypoplastic but most often hyperplastic, is sometimes clearly differentiated, with a practically normal structure. It is, however, not functional; there is no glycogen formation in the layer of intermediate cells, and the Schiller test is iodine negative. From this point, there are two possible developments:

a) either the metaplastic squamous cell epithelium will spontaneously become iodine positive after a certain lapse of time,

b) or the epithelium will remain iodine negative, testifying to a disturbance in its function. This disturbance may be simple, as for instance the non-formation of glycogen, but it may also be evidence of a benign architectural and cellular disturbance, such as dysplasia, or even as severe as in carcinoma, as we shall show later.

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AN EROSION (Latin: erodere, eradere = gnaw off) is a point-like or plain-like defect of an epithelial integument. The erosion of the portio vaginalis uteri (ectocervix) is found circularly around the external os of the uterus, at one or both lips of the os uteri. The change originates from inflammatory, mechanical or chemical irritations. In the typical case the pavement epithelium is gnawed off up to the basement membrane (membrana propria). Such injuries can heal without scars. If the basement membrane is destroyed by the defect, we see an ulcer. Ulcerations always heal with scar formations. The true cervical erosion (erosio vera) is at the most found in 10 per cent of the cases of inflammatorily changed cervices.

We have an ectopy (Greek: ἐκ τόπος = located out of the place) if mucus-producing cervical cylindric epithelium grows down on the "terrain" of the portio. This may occur, for instance, if first an erosio vera has come into being by cervical hyper-

secretion. This defect can be covered by regeneration and proliferation of the squamous epithelium. If this does not take place, cervical epithelium can grow down on the terrain of the erosion. It grows monostratified, at most forming little flat glands and fills up the whole erosion. This is the classical case of an ectopy.

Robert Meyer termed this process the first stage of the healing of the erosion. Today one also speaks of "descending epidermization." Of course, the erosion is a pathological change. It is my opinion that one should also designate the form of ectopy described above as pathological. At best one can speak of a defective attempt of healing. The inflammation is pathogenetically part of pathological processes, even if it follows physiological models and results in healing. The ectopy, however, does not even result in healing, but only brings about the covering of an epithelial defect. The fact is that epithelium of the cervix uteri leaves its matrix and settles at another place (ἐκ τόπος). If the now existing ectopy would mean a final state, then the designation "healing" would be, up to a certain degree, applicable. As the portio in this changed kind, however, is bound to injure the vaginal milieu already by hyperproduction of alkaline mucus, there is no rest, and this is not healing. I believe this statement to be necessary for purposes of definition and of practical clinical use.

One should still add that for the most part the subepithelial tissue of erosion and ectopy, so to speak the fundament of the two types of epithelium, is not changed in its construction of fibers and the amount of fibers to the epithelium. One can term the so-called juvenile erosion, congenital ectopy, though there is a genetically explainable shifting of limits between cylindric epithelium and squamous epithelium including the accompanying "substructure."

The ectropion (Greek: ἐκτρέπειν = turn) has originated in most cases from laceration. I think that most of the changes of the os uteri can originally be reduced to an ectropion. The mechanism of origin can be simply explained: During labor or abortion the cervix is dilated and disturbed. Frequently there occur, not only by pressure but also by traction, cicatrizating lacerations of the muscular and elastic fibrous connective tissue and epithelium is disturbed. The turning up of cervical epithelium, together with the underlying fibrous tissue, at the cervical rupture is evident. But also without rupture an ectropion can occur. The mechanism is possible in two ways.

As is histologically demonstrable, there are muscular fasciculi in the cervix uteri, which lie circularly lengthwise in the periphery of the cervical musculature. They nearly always send off very strong muscular fasciculi up to the subepithelial tissue of the external os. Between these fasciculi and the cervical canal networks of smooth and elastic fibers are found which also extend to the top of the ectocervix. This muscular plexus can be injured especially during labor. During the period of regression it is partially hyalinized and indurated. Processes of retraction of the peripheral muscular fasciculi then draw the lower parts of the cervix outwards to the terrain of the portio, because a compensating traction of the remaining musculature may be wanting: The ectropion of cervical epithelium with accompanied subepithelial fibrous tissue—and often hyaline cicatricial tissue—exists. However, the normal boundary between the cylindric epithelium of the cervix and the squamous epithelium of the portio at first is not even disturbed. The plane of the external os lies deeper than before and thus outside on the portio.

The second possible mechanism is represented by subepithelial cicatrization. If the peripheral muscular fasciculi are not adequate to effect a muscularly caused protrusion of cervical epithelium, then the scar

retraction of fibrous tissue in the cervix can prevent the invagination of the cervical epithelium everted by the process of labor or abortion. Also from this an ectropion remains. I would like to call these three changes at the os uteri (erosion, ectopy and ectropion) the primary injuries of the os uteri. However, this definition has to be used for the ectopy with reservation for the reasons mentioned above.

All three changes can cause secondary conditions. The erosio vera can become epidermized with descending cervical epithelium and ascending vaginal squamous epithelium. Ectopy and ectropion can become secondarily eroded. All three pictures can be and can remain inflammatorily changed.

The possible alterations of the epithelial integuments are very various. The most frequent change is the so-called epidermization. The term is derived from the histological structure of the skin. Naturally it has nothing to do with the ectocervix. On one side one wants to express that an epidermization by squamous epithelium takes place and on the other that this squamous epithelium shows a skin-like degree of hardness, as is otherwise not found at the portio. The process takes place in two ways.

The first type is the ascending epidermization. Squamous epithelium of the vaginal membrane grows over cervical cylindrical epithelium on the top of the fold of mucous membrane, for instance at the ectropion or grows under this epithelium in the singular glandular lumina, always following the tunica propria and lifting off the cylindric epithelium. There occurs the apparent formation of cones of squamous epithelium in the former cervical glands and also bridge-like covering and occlusion of them with formation of ovula nabothi. Mostly, all this is accompanied by chronic inflammation. These pictures have been described classically by Robert Meyer. There is little to add, but I have objections against the term of the second stage of the "erosion healing." This so-called ascending epidermization is not a healing process, but a pathological one. The organism has only fixed possibilities to respond to irritations or changes. Here, for instance, is the proliferation of squamous epithelium, which strives to occupy its old place, but has to struggle with cervical epithelium. This zone of transformation, designated according to Hinselmann, is not a physiological product, but a pathological one. No one would designate the inflammatory-proliferative change of a liver cirrhosis as healing. The formation of inflammation tissue in this case is also a reaction of the organism. The injury is considerable; one cannot talk of healing, unless one is able to stop the process.

It is quite a different question as to whether or not all epidermization processes must have a poor prognosis.

The second type of epidermization is the direct transformation of cervical epithelium at ectopy and ectropion according to the type of metaplasia. In the course of numerous cell generations cylindrical epithelium becomes anaplastic and transformed into the next lower degree of differentiation, the squamous epithelium. Epithelial changes of this kind, which can be observed in many different stages, and the mechanism of origin which is known also by processes in the epithelium or connective tissue at other sites in the organism, can be explained even less to be healing processes than the above described. There is no doubt that so-called epidermized cervices nearly always show chronic inflammatory changes, deep in the tissue (periglandular or subepithelial), and that they can frequently be eroded freshly at the surface.

CARLO SIRTORI

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IN THE CASE of ectopy, the endocervical cylindrical epithelium takes the place of the ectocervical squamous epithelium. The histological diagnosis of ectopy is only presumptive and can be postulated when squamous epithelium of the ectocervical type with cervical gland openings is observed.

Ectropion is characterized by a fibropapillomatosis of the cylindrical surface epithelium; in the chorion, lymphoplasmacellular elements are generally present.

Epidermization is characterized by a basal cell hyperplasia of the endocervical epithelium, which during the next differentiation, takes the features of the squamous epithelium, without reaching the high degree of keratinization peculiar of the ectocervical epithelium.

Discussion

F. A. Langley, Manchester, England, U.K.: I find the descriptions given here of the histomorphology of ectropion, ectopy and erosion confusing. It is probably because the authors have approached the discussion theoretically and because of slightly different national usages of the terms. In the "naked eye" specimen it is possible to distinguish between erosion and ectropion but the distinction is lost under the microscope. The term erosion, like so many in pathology, no longer retains its original meaning but when speaking of the cervix means that squamous epithelium has been replaced by endocervical epithelium. Figure 1 shows a beginning erosion. The squamous epithelium is being lost by ulceration and replaced by columnar epithelium from adjacent glands. I would not really regard the endocervical epithelium as ectopic in an erosion.

It seems doubtful if the ascending or descending type of epidermization described by Korte is of frequent occurrence. Although it might be expected to occur by analogy with, say, the healing of an injury of the skin. Examination of the late stages of "healing" of an erosion may support such a conclusion but the study of early stages seems to indicate that epidermization usually begins as "reserve cell" hyperplasia, as described by de Brux.

Rudolf Ulm, Vienna Austria: Our conception of the pathological conditions in question agrees, to a large extent, with the description given by the main authors. A short summary of our opinion will show where discrepancies prevail.

We understand ectopy generally to mean the unusual localization of cylindrical epithelium outside the external os, on the surface of the ectocervix, where normally only squamous epithelium is found. This condition develops by the growing of endocervical mucosa over a true erosion. Depending upon whether such an ectopy is studied histologically shortly after its development or only in the stage of epidermoidal healing, the histomorphological picture will be different. In the former case one will find endocervical mucosa over a chronically inflamed and infiltrated mesenchymal base; in the latter case a superficial covering of squamous epithelium is only here and there interrupted by islets of cylindrical epithelium around glandular openings. Usually an inflammatory connective tissue reaction will already be absent in this stage.

The ectropion arises by eversion of the outermost parts of the endocervical canal. The endocervical epithelium has not left its original connective tissue base. It has not grown beyond its original border but has been displaced together with its underlying tissue from the endocervix to the surface of the ectocervix. Therefore, the histomorphological picture will not be different from that of normal endocervical mucosa.

We understand epidermization to mean the replacement of a cylindrical epithelial covering by squamous epithelium. This may occur either by overgrowth from the margins or by squamoid mataplasia, as for instance in the case of a reserve cell hyperplasia. The histomorphological picture corresponds in all details to the one seen in a healing ectopy.

Closing Remarks

Wolfgang Korte: I thank Langley for his discussion. I fully agree with his opinion that it is also possible to distinguish between erosion and ectropion with the naked eye. Histomorphologically, however, one should keep to the well discernible separation of the four mentioned terms, which can be done in the histological preparation, too. I do not think it is very suitable to name formal pathogenetically known states of development and final

FIG. 1. Haematoxylin and Eosin x12. Beginning cervical erosion.

conditions of injuries of the os of uterus indiscriminately with the clinical collective noun "erosion."

I want to clear up the term "epidermization." I only have dealt with the descending epidermization by cylindric epithelium on the ectopy, but not with descending epidermization. I am able, however, to show many preparations for the ascending epidermization.

Carlo Sirtori: According to Ulm and Langley's suggestions, we can state as follows: "ectopy" means substitution of the ectocervical squamous epithelium by the endocervical cylindrical epithelium. Ectopies can be due: (a) to an ulceration of the ectocervix which is repaired by cylindrical epithelium instead of squamous epithelium: (b) to a congenital malposition of the ectocervix, so that cylindrical and squamous epithelia are intermingled; (c) to a prolapse-like displacement, of the endocervical tissue which invades the ectocervix. This displacement could be called more properly, "ectropion." Generally, it provokes papillous-like patterns of the endocervical epithelium, due to edema and congestion of the connective tissue. The ectopies Type (a) can be recognized microscopically by the presence of an inflammatory reaction in the connective tissue; the ectopies Type (b) do not show inflammatory reactions or papillae. As far as epidermization is concerned, I think it is always a proliferating process arising from the basal cells.

These etiopathogenetic and histological classifications have no absolute value, but can be useful as a pathway for practical purposes.

Histochemistry of Ectopy, Ectropion and Epidermization

JEAN BERGER

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IF ONE UTILIZES histochemical staining methods for the evaluation of epithelial alterations of the cervix and endocervix, one should critically recognize the value of these methods.

In gynecological histopathology, histochemistry is very useful. It allows the specific demonstration of morphological structures and solves histochemical problems in regard to normal and atypical growth processes. Furthermore, hormonally influenced functional performances of the

TABLE 1

	No. cases	RNA	DNA	Acid phosphatase	Phospho-amidase	Glycogen	Neutral mucopoly-saccharides	Acid mucopoly-saccharides
Normal epithelium	14	+	+	+ basal	(+)	+++	+++	
Regenerative epithelium	8	++	+	++	++	+	++	
Atrophic epithelium	26	+−	+−	(+)	(+)	(+)	+	
Simple atypical (Hinselmann I) epithelium	59	+	+	+	+	(+)	+	
Simple atypical epithelium with plugs (Hinselmann II)	86	++	+	+	+	(+)	+	
"Unruhiges" epithelium	12	++	++	++	++	(+)	+	−
Hinselmann III	16	+	+	++	++	(+)	(+)	(−)
Hinselmann IV abc	22	++	++	+++	++	((+))	(+)	−
Invasive carcinoma, not cornified	11	++	++	+++	++	−	−	(+)
Invasive carcinoma, cornified	3	+	++	+++	+++	(+)	+	++

cellular and tissue metabolism can be studied.

We have studied more than 300 cervical biopsies histologically and histochemically, using adjacent sections of the same block for the various studies. The following histochemical staining procedures and reactions have been used:

1. Demonstration of the ribonucleoproteides (RNA) and the desoxyribonucleoproteides (DNA) by methyl-green-pyronin staining after Brachet. This is, however, not a specific histochemical reaction, as already stated by Brachet and Taft,[1] since mucoproteides stain the same way as the RNA, i.e., orange-red. More important is the ribonuclease digestion of Brachet which we employed in all cases.

As an additional test for all nucleoproteides we used the toluidine blue reaction. We are then able to demonstrate the cyanophilia in particular.

2. The acid phosphatase has been studied in each case by the Gomori technic. The enzymes studied in this way play an important role, especially in nucleic acid metabolism and the synthesis of the carbohydrates.

3. The phospho - amidase - reaction has been employed. This is very intricate, since it depends very much on exact incubation time, temperature, etc.

4. For demonstration of the polysaccharides the following staining technics have been applied:

a. Glycogen staining after Bauer, b. McManus-Hotchkiss reaction (PAS reaction) for demonstration of the neutral mucopolysaccharides, c. The alcian-blue stain after Ebner and Lindenschmidt, which represents a selective staining for neutral and acid mucopolysaccharides.

5. Disulfide bonds (S-S Cystein) have been demonstrated by the Chèvremont-Frédéric reaction.

6. Sulf-hydril compounds (SH-SH) like cystin could be shown with the aid of the method after Pearse.

7. Further stains (however no histochemical reactions in the proper sense) have been applied supplementarily, such as hematoxylin-eosin, azan after Heidenheim, silver impregnation after del Rio-Hortega, and a combined staining technic which consists of polychrome stain (Papanicolaou)

and silver impregnation (Hortega) as devised by ourselves.

From Table 1 it can be gathered that certain reactions and positive stains for given substances are significant for specific epithelial changes. In this group the demonstration of DNA, RNA, acid phosphatase, phosphoamidase, glycogen and the neutral mucopolysaccharides can be listed.

It also can be recognized from the Table that in all incidences of an increased growing activity, i.e., in the basal layers, atypical

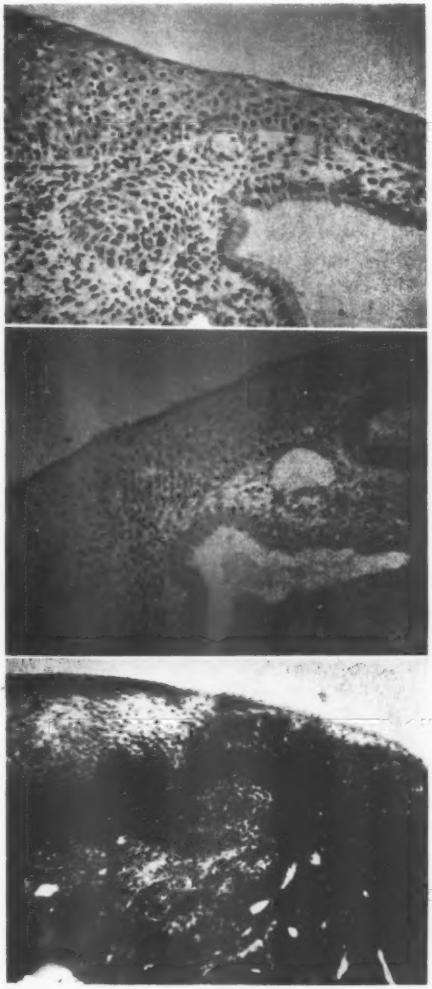

FIG. 1 (Top). Ectropion and beginning cornification in hematoxylin-eosin. FIG. 2 (Center). Methylpyronin reaction in the section, RNA shows red and DNA blue. FIG. 3 (Bottom). Demonstration of acid phosphatase in the same tissue part; the acid phosphatase is stained black.

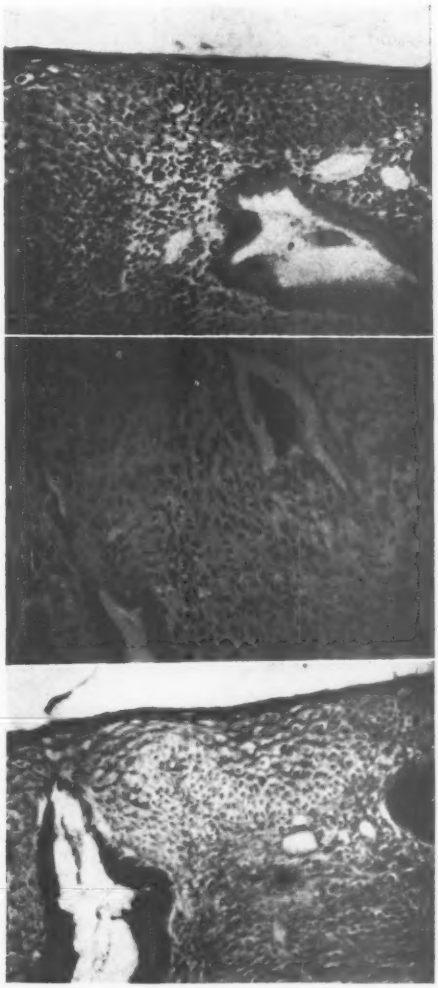

FIG. 4 (Top). PAS staining in same section. FIG. 5 (Center). Detail from former figure: PAS positive substances extending towards parabasal layers. FIG. 6 (Bottom). PAS-alcian blue stain of same case: neutral mucopolysaccharides stain red, while acid mucopolysaccharides stain in blue-purple.

epithelium, regenerative epithelium and carcinoma with increased proliferative power, the values of RNA, DNA, and acid phosphatase activity are increased. In a normally functioning epithelium (as normal epithelium or slightly inflamed epithelium) the glycogen, or the neutral mucopolysaccharides respectively, can be demonstrated in normal quantity and distribution. If the function is minimally disturbed, this is not still possible. The demonstration of S-S and SH-SH groups has an importance in regard to the determination of the degree of the cornification.

Histochemical Reaction on Ectopy, Ectropion and Epidermization: We shall explain the histochemical stainings and reactions by a typical case. Here it can be definitely demonstrated that increased growing tendency, like in epidermization of the ectopy by the basal cells of the squamous epithelium, is combined with augmented RNA, and DNA, acid phosphatase and phosphoamidase. After a certain differentiation has taken place, glycogen or the neutral mucopolysaccharides in the lower layers become demonstrable. Figure 1 shows overgrowing of a gland by a normal squamous epithelium in hematoxylin-eosin stain. Figure 2 shows the very same tissue portion in methyl-green-pyronin stain. It discloses the increased content of RNA and DNA within the growing zone. In Figure 3 we see unequivocally the increase of the acid phosphatase at the site of increased activity, i.e., basally in the growing zone. The glands are covered and are only visible faintly. Figures 4 and 5 are pictures of PAS neutral mucopolysaccharides reactions of the same case. The red staining precipitates are visible in the already differentiated upper cells in small quantities. Figure 6 depicts more clearly the distribution of the neutral and acid mucopolysaccharides, stained by PAS—alcian blue. The red parts consist of neutral mucopolysaccharides; the blue-purple parts show the presence of acid mucopolysaccharides. The blue-purple basal membrane which consists of acid muco-proteids is clearly visible.

Conclusively we may state that histochemical staining technics especially in epidermizations may give valuable information about regeneration and beginning differentiation. A better differentiation by demonstration of individual substances, such as RNA or acid phosphatase or the demonstration of the polysaccharides may reveal whether a normal regeneration is taking place or whether the regeneration is finished. Keeping in mind the importance of an undisturbed regeneration, especially in regard to a possible future dedifferentiation or carcinogenesis, the role of histochemistry must not be underestimated.

ALVAN G. FORAKER

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VARIOUS studies performed in our laboratory[1] have shown that the reactions of squamous mucosal cells in the cervix vary with growth or maturation. These are the important controlling factors, more so than position, "benignancy," etc. When metaplastic or ectocervical squamous cells are exposed to air and trauma by ectropion,[2] keratinization occurs. In this process, the sulfhydril group of cystein is oxidized to the disulfide bond of cystin. The process of keratinization is also accompanied by

marked increase in cytoplasmic mass density, which can be observed by interference microscopy[3] and by soft x-rays.[4] Proliferating cells in ectocervical mucosa epidermidization have dehydrogenase activity. With maturation, this diminishes.[5] In the past ten years we have applied more than twenty special cell technics to the investigation of cervical mucosa. None has sharply delineated normal epithelium from metaplasia from dysplasia from intraepithelial from invasive squamous cell carcinoma.

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Exfoliative Cytology of Ectopy, Ectropion and Epidermization

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BY ECTOPY we understand a change which can be diagnosed either by histology or by colposcopy. It is characterized by the fact that the squamous epithelium of the ectocervix is replaced in varying degree by the mucosa of the endocervix. If there is only an extroversion of the cervical canal and if therefore the mucosa of the cervix becomes visible, we call this an ectropion. In this connection by epidermization we understand the healing process of ectopy respectively ectropion, that is, the replacement of the dystopic columnar epithelium of the ectocervix by squamous epithelium.

Because of the anatomical changes in ectopy and ectropion we expected to find more exfoliated columnar cells than average. The permanent comparison of the colposcopical findings with the cytological picture seemed to confirm these expectations. In order to clarify whether this impression was justified or not, we compared the number of the exfoliated squamous cells to the number of exfoliated columnar cells, employing 100 cases with colposcopically normal ectocervices as well as 100 cases of colposcopically ascertained ectopy or ectropion. In the same way we established the relation of squamous cells to columnar cells in 100 additional cases in which the epidermization of an ectopy or an ectropion, respectively, was completed, that is, the entire ectocervix being again covered by the squamous epithelium. Colposcopically such "transformation zones" are recognizable on the one hand by the quality of the squamous epithelium which differs from the norm, and on the other hand by still open ducts of cervical glands or retention-cysts (ovula nabothi).

Cytologically prominent findings are to be expected in epidermization, if the newly formed squamous epithelium shows essential differences compared with the normal squamous epithelium. However, a more intimate study of the cytology of the

TABLE 1

	Squamous cells	Columnar cells
Ectopy and Ectropion	15412 (77%)	4588 (23%)
Epidermization	16888 (84%)	3112 (16%)
Normal Ectocervix	18596 (93%)	1404 (7%)

single forms of the pathological epithelium will be discussed later in this journal. The evaluation of the relation of squamous cells to columnar cells was accomplished in these cases in order to throw light upon differences from ectopy and ectropion respectively.

The 300 smears were taken according to the scraping method. In each case 200 cells were counted and defined either as squamous cells or as columnar cells. Finally the results of the 100 cases of the respective single groups were summed up and thus the total number was ascertained. (Table 1.)

For ectopy or ectropion respectively, as well as for the normal ectocervix, the results correspond to the expectations. In ectopy columnar cells were found more than three times as often as compared with normal cases. We were, however, surprised by the relatively large number of columnar cells in epidermization, since—as already mentioned—only cases with complete epidermization were under examination. According to the findings that here the entire ectocervix is covered again by the squamous epithelium, we expected a result corresponding approximately to the normal ectocervix. To some extent, the result achieved may be explained perhaps by the fact that columnar cells from the cervical glands reach the surface through open ducts of the glands.

As for the rest, the relation of squamous cells to columnar cells considered in single cases certainly does not correspond always to the total result. Thus the 200 cells counted in each of 12 ectopies proved to be exclusively squamous cells. In the remaining cases the share of columnar cells varied from one to 188. Also in the normal ectocervix columnar cells could often be found in large numbers—192 in one case. For the result of the single case, therefore, other factors, such as inflammations, traumas of various kinds, hormonal influences, etc., are without any doubt, of importance.

Generally, however, we can presume on the strength of the total result, the frequency or the absence of columnar cells in the smear enables us to draw a cautious conclusion as to the appearance of the cervix: many columnar cells point to ectopy or ectropion respectively; their complete absence induces us to expect a normal cervix.

Here, of course, we have to mention that these questions are decided more expediently by the help of colposcopy, that is, with absolute certainty at a quick glance.

JEAN DE BRUX, JACQUELINE DUPRÉ-FROMENT

Paris, France

IT IS LOGICAL to include both the ectropion and the ectopy in the same study, for not only are their smears nearly similar, but also each may undergo modification as the consequence of a metaplastic process. Furthermore, in certain cases the ectopy results from the incomplete epidermization of a large ectropion.

The diagnosis of ectopy is based on the presence of a large area of columnar

FIG. 1. Ectropion: Hyperplastic endocervical cells.

FIG. 2. Ectropion: Hyperplastic endocervical cells in larger magnification.

epithelium on the ectocervix, which normally is covered by squamous epithelium.

The term ectropion signifies the eversion, at the external orifice of the cervix, of the endocervical mucosa, usually in this case hyperplastic.

As thus defined, these two lesions are evidenced on the cervical smear by the presence of more or less numerous endocervical cells. Schematically, three sorts of criteria permit their diagnosis:

1. The abnormal localization of the cells, manifested by the presence of elements of an endocervical type on an ectocervical smear, which normally is composed solely of squamous cells, of a few junctional cells, mucus and polynuclear cells.

2. A quantitative factor, in relation to the abundance of these cellular elements.

3. A qualitative factor represented by the hyperplastic character of these cells, to which is often added a beginning metamorphosis owing to their unusual location which puts them in contact with a vaginal medium which is more or less irritating.

In the normal state, the endocervical

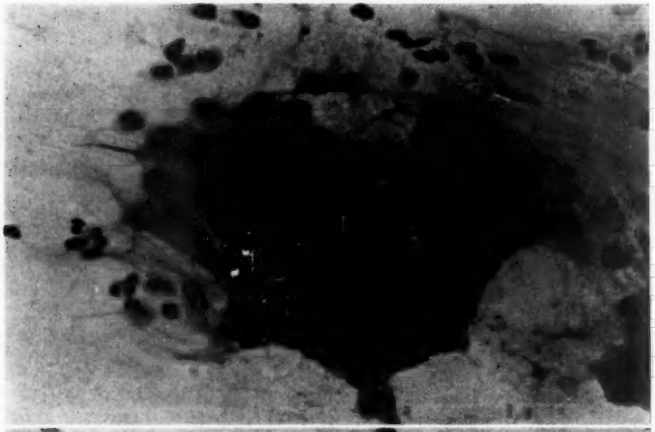

FIG. 3. Cluster of hyperplastic endocervical cells displaying anisonucleosis.

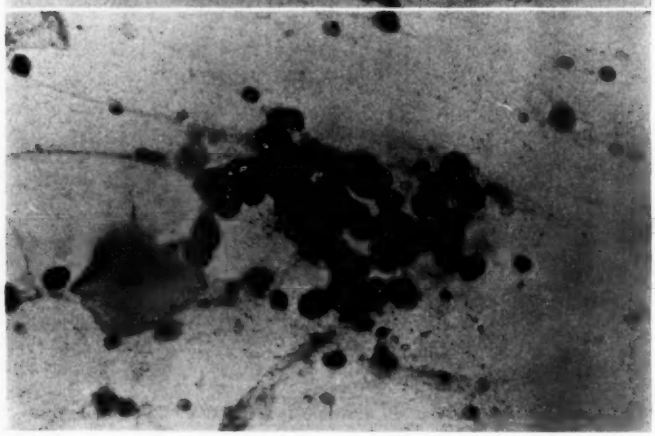

FIG. 4. Naked hyperplastic endocervical nuclei with alveolar aspect.

cell is an element measuring 15μ-20μ, whose profile view shows a cylindrical form with the nucleus situated at one end, or which, if observed from the "front," appears approximately round, with a central nucleus. The cytoplasm is cyanophilic, homogeneous, and rather limited; the nucleus is round, regular, surrounded by a fine membrane, and its chromatin is delicately pigmented and nucleolated (Fig. 1).

In the hyperplastic state the cell keeps the same aspect, but its structural details are accentuated: the nucleus is larger, the outline of its membrane is finely emphasized, the chromatin dots are denser and more visible, and the nucleolus grows and appears in relief. The cytoplasm spreads and becomes more cyanophilic, sometimes containing microvacuoles; its contours become more distinct, and the extremity opposite the nucleus rounds out (Fig. 2).

More rarely, the chromatin web is distended by the hyperplasia, and its meshes are separated by what appear to be regularly-distributed vacuoles. In some cases, the structure of the nucleus becomes simplified to the extreme, reduced to a few grains of chromatin, all of equal size, stuck against the nuclear membrane, whereas the nu-

cleolus, thus isolated, becomes very prominent; however, this aspect is rare (Fig. 3).

These special forms may sometimes be difficult to distinguish from certain cancer cells with sparse chromatin; but in the latter, the chromatin grains are thick, unequal in size, and, especially, irregularly distributed.

The endocervical elements described above are isolated. Very often they appear in fringes, even having conserved their ciliated border, but usually they are found in small groups or in moderate-sized patches.

At first sight, the groups seem to consist solely of nuclei packed together, somewhat resembling marbles because of their equal volume and regular contours. The border of the group or patch must be examined in order to recognize the cylindrical form of the cytoplasm; in the surrounding area, naked nuclei are dispersed, but are always in small number (Fig. 4).

On an ectocervical smear it is impossible to distinguish an ectopy from an ectropion, the shades by which they differ being extremely slight: in the ectopy the disposition of the cells is rather in the form of fringes or small groups, whereas in the ectropion the elements may form relatively

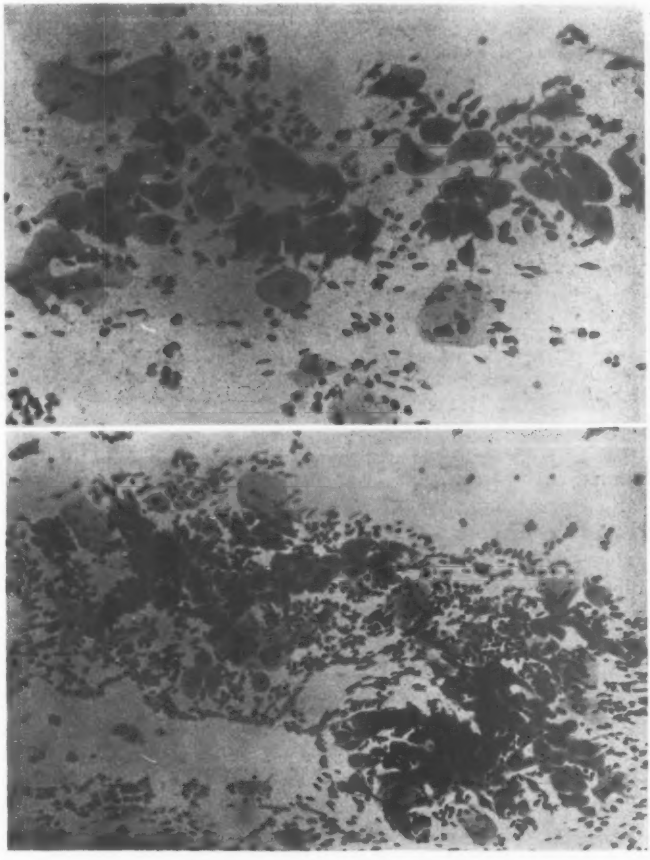

FIG. 5. In the center, transformed endocervical cells; at right and left, so-called metaplastic parabasal cells.

FIG. 6. So-called metaplastic parabasal cells among which there are some clusters of transformed endocervical cells.

FIG. 7. Metaplastic cells displaying anisonucleosis and slight nuclear irregularity, vacuolization of the cytoplasm and even macrophagia.

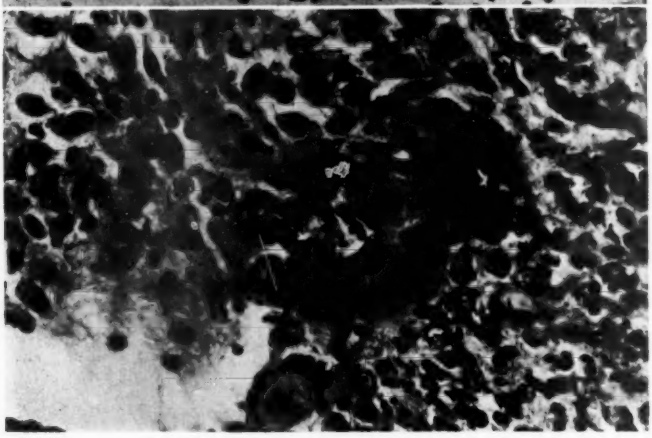

FIG. 8. Histological section from the same site as the elements of the previous picture. Metaplastic endocervical cells in the process of exfoliation.

large patches, sometimes folded over, and limited peripherally by a layer of cylindrical elements arranged in palisades. Certain of these groups may assume an alveolar aspect owing to the retraction of the nuclei, whose contours are more or less angular, whereas the cytoplasmic limits become linear.

An examination of an endocervical smear, however, permits a certain discrimination, since, if the sampling technic is good, the smear appears normal in the case of an ectopy, whereas in the case of an ectropion it often presents an abundance of hyperplastic endocervical cells.

Such is the aspect of the ectopy and of the ectropion in the "pure" state. In reality, however, these lesions are nearly always modified, either by a simple metamorphosis of their border elements (particularly in the case of an ectropion), or by a true process of metaplasia following the multiplication of the reserve cells. Hence, in addition to the cellular patches above described, we must also recognize other formations, namely, "altered endocervical cells" and "metaplastic cells" or "parabasal cells":

(a) In the case of the altered endocervical cells, the endocervical cells in contact with the vaginal medium will be trans-

formed in place, that is, become metamorphosed. There is then noted the presence of small shreds of tissue, often stretched, sometimes even tattered, composed of contiguous cellular elements whose morphology is vague, midway between the columnar cell and the basal type of squamous cell. The general form of the cytoplasm is still elongated, even stretched at the border of the clump, but is more spread out, and certain of the cells are rounded or polygonal. The nuclei, voluminous and very regular, are tinged with purple, whereas the cytoplasm tends to become polychromatophilic (Fig. 5).

(b) The metaplastic cells present a very characteristic aspect. They form groups in which they are agglutinated rather than contiguous, as if they had exfoliated not in strips but singly; at the interior of the patch they are often superimposed. Their size is that of an external basal cell, rounded or rather oval, with one or two fusiform extremities. Their cytoplasm is definitely polychromatophilic, with regular, hyperplastic nuclei; but its color is purplish, and it may become elongated like its surrounding cytoplasm. In this case it develops a central or para-central groove (Fig. 6).

When the metaplasia is more organized, the cells appear isolated from one another, dispersed, or grouped in bands. They are rounded or navicular in form and larger than in the former case. Their cytoplasm is often vacuolized and peripherally limited by a darker homogeneous sediment. These elements belong to those called "junctional cells" of the ecto-endocervix, and whose entity is more theoretic than real, if one considers the marked tendency of this frontier zone to become metaplastic (Fig. 7).

Inflammation of the ectropions is very frequent and may be manifested by cellular anomalies which, without constituting true dysplasias, sometimes render a diagnosis difficult. We refer here essentially to those cases in which there appears, in the midst of the group, a rather marked anisonucleosis which, added to a particularly active hyperplasia, is with difficulty distinguished from certain immature epidermoid epitheliomas with very regular nuclei. In these cases the chromatin must be thoroughly studied in order to discover the irregularities of its distribution (Fig. 8).

Conclusion

The diagnosis of ectropions and of ectopies uncomplicated by dysplasia offers little difficulty and can be made almost solely on the basis of the coexistence on the same smear of hyperplastic endocervical cells and "altered" or metaplastic cells.

The clinical context is another diagnostic aid if one considers the frequency of a high estrogenic stimulation expressed by the smear, and the frequency of ectropions occurring during pregnancy, for instance. It is true, moreover, that these lesions represent absolutely normal reactions to trauma and inflammation, but they are nevertheless of extreme importance, for they constitute the starting point of all the dysplasias and perhaps furnish the "pretext" for the formation of the cancers.

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ECTROPION, or ectopy, is the result of a cervical laceration through which the swollen and congested mucosa "rolls out" from the cervical canal, producing an appearance similar to, but not quite identical with, cervical erosion.[1] By epidermization

of the cervix we mean the replacement of these exposed areas of the cervical mucosa normally covered with columnar cells by squamous cell epithelium usually encroaching upon this area in the form of long, slender epithelia tongues made up of basal and parabasal cells. Another theory to explain cervical epidermization is through the process of metaplasia as it is observed in various mucous membranes lined by secretory or columnar cells under the effect of chronic irritation. It is our opinion that in the cervix both processess can occur and that it is difficult indeed to separate them. It usually occurs in middle-aged women in whom repeated birth trauma to the cervical canal has caused multiple cervical lacerations and inadequate scar formation. It is rarely encountered in nulliparas, in whom it may be produced by the wearing of improperly fitted pessaries. Epidermization of an ectropion or a cervical erosion never reaches the protective thickness of the squamous cell layer covering the portio vaginalis.

The cytological picture of cervical ectopy is characterized by the increased appearance of basal and parabasal cells as well as of clumps of endocervical cells together with an increase of inflammatory cells. The epithelial cell of cervical epidermization is usually of the parabasal or intermediate cell type and has also been called "spider cell" by Ruth Graham.[2] Sometimes these young cells are loaded with glycogen, which leads to the appearance of the koilocytotic cells of Koss.[3] Together these cells dominate the picture in the cervical scraping taken from an ectopic area with epidermization. Cervical scraping or endocervical aspiration usually demonstrates the great vulnerability of these types of lesions, which bleed easily on touch. This can be explained by the fact that the subepithelial capillaries are usually dilated and not very well protected by the thin layer of metaplastic epithelium. Dyskaryotic cell changes in this epithelium justify a classification of a Class II Papanicolaou smear, but rarely are the changes pronounced enough to be suspicious of malignancy. We definitely feel that cells from these lesions can and should be differentiated from cells with premalignant dyskaryosis, and we definitely do not regard this lesion as premalignant.

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EKKEHARD KOFLER, HANS KREMER, RUDOLF ULM

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USING the direct smear technic with aid of a platinum loop or an Ayre spatula, one will find in the smear of an ectopy or an ectropion columnar cells which are either lying singly or grouped in palisade-like or mosaic formations. The cells are distinguished by relatively large, pale, eccentricly situated nuclei. The frequently found degenerating cytoplasm seems stretched in the palisade formation. Fresh ectopic processes are marked by an infiltration of leukocytes of differing intensity while in long standing ectropions lymphocytes and plasmacells constitute the majority as an expression of chronic inflammatory processes. Naturally besides these cell elements one will find squamous epithelia and often large amounts of mucus.

In an epidermization one finds basically the same celluar elements as in the ectopy. Frequently, however, cells from the deeper layers of the epithelium—basal and parabasal cells—appear. Moreover, one sees more or less pronounced atypias in the squamous epithelium. Cytologically it may sometimes be difficult to evaluate cells from an epidermization, since as an expression of the highly increased proliferative activity they may fake atypias. Thus even the experienced cytologist may occasionally make a false positive diagnosis. This is unavoidable, since the cytologist has only single cells or groups of such altered cells available for evaluation. Only histology can decide between "epidermization with atypias" or "already malignant degeneration" by reviewing the entire tissue structure. But even for histology this decision is often not an easy one.

Discussion

Werner Bickenbach and Hans-Jürgen Soost Munich Germany: It was interesting to find from a series of comparable examinations (as Bajardi did) to what extent endocervical cells exist in smears from ectopies, transformation zones and normal ectocervices. The results prove that it is impossible to diagnose the ectopy simply by the cytological smear. In our opinion this is actually not necessary, since it can be done more easily and better by colposcopy.

However, the study of endocervical cells is important for the differential diagnosis respective to malignant cells. The columnar cells are mostly poorly preserved and the nuclei are often entirely bare of cytoplasm, if the smear is not taken by direct scraping with a spatula. Sometimes the typical cylindrical form or the settling of the cells in palisades is preserved in a few groups.

In addition to the columnar cells, the different forms of which have been described by the main authors, parabasal cells appear to a lesser or greater degree in ectopy and epidermization. One can also find cells, the morphologic picture of which is between endocervical and parabasal cells, which are often especially difficult to interpret. They lie together, mostly in groups or in a tissue formation. We think that the reference to this by de Brux is remarkable and we also are of the opinion that these cells and cell-groups of different forms are being exfoliated or scraped off from areas of epidermization and metaplasia.

Marcel Gaudefroy, Lille, Nord, France: I agree with the main authors on the exfoliative cytology of ectopy, ectropion and epidermization. I would like to add that I am not surprised that Bajardi finds a relatively large number of columnar cells in epidermization in his counting of squamous and cylindrical cells. In my opinion, this fact is observed not only because of the presence of open cervical glands at the surface of the ectocervix, but also, as is pointed out by von Haam, because the epidermization is produced by slender epithelial tongues incompletely encroaching on the ectocervix. It is easy to verify this process by colposcopic examination after the Schiller test.

Also, it is my opinion, similar to that of de Brux and von Haam, that the differential diagnosis from malignant cells should be made in almost all cases. Important nuclear changes in shape and in size are frequently observed, but the chromatin material remains smooth and uniform, and not in gross clumps.

Wolfgang Korte, Bonn, Germany: Already the real catarrh of the cervix causes an increase in the amount of cervical epithelial cells. The chronic inflammation of cervical epithelium with all possible cellular alterations provides suggestive findings.

I fully agree with the report of de Brux and Dupré-Froment. Also, I am of the same opinion as von Haam that epidermization can occur as replacement and as metaplasia. Both alterations are attempts at healing, but are certainly pathological processes. I do not know any ectopy or ectropion with processes of epidermization, which would not simultaneously show true erosive lesions at the epidermization epithelium. One always finds increased endocervical cells in the smears of ectopies and ectropions, which are not quite fresh, often with simple dyskaryosis and very frequently so-called erosion cells, i.e., basal, parabasal and intermediate cells of the epidermization epithelium. In addition, these cell forms are in most cases altered by inflammation and also by mechanical trauma.

One will not be able to separate ectopy and ectropion from the vaginal smear alone without colposcopy and viewing the macroscopic aspect. I nearly always restrict myself in the cytological report to a summary and to a description which is understandable for the noncytologist.

Hans Muth, Muenster/Westfalen, Germany: We found endocervical cells more frequently than Bajardi. We have found them in 40 per cent of the 478 cases of ectopies examined colposcopically and in 25 per cent of 1,054 cases of transformation

zones. While with the colposcopical diagnosis of ectopy, atypical cells were associated only rarely in the vaginal smear (1.6 per cent), we found in 6 per cent atypical cells in the cytological smear in the cases with the colposcopical diagnosis of regenerative epithelium. Among the latter in 10 per cent of the cases a preclinical carcinoma could be demonstrated. In all of these cases, however, an atypical transformation zone (Glatthaar, Wespi) was present. Thus, the cytological examination is a necessity.

Guillermo Terzano, Buenos Aires, Argentina: It is generally admitted that those conditions of the cervix called ectopy, ectropion and epidermization (satisfactorily explained by Robert Meyer as lesions of an inflammatory nature), can be considered similar to the process of instability of the endocervical and the ectocervical epithelia during the last weeks of intra-uterine life.

To try to describe schematic differences of these lesions by means of exfoliative cytology is not a simple matter. The number of desquamated cells and the percentages of each type of cell (squamous and columnar) in smears vary in vaginal aspirations, cervical aspirations, cervical scrapings or direct contact smears; therefore, it is logical to observe what is shown in Table 1 in Bajardi's paper. The cytological picture sometimes does not differ from that of chronic cervicitis, and also, confusing cellular patterns may appear if associated or if concomitant factors are present.

We may say, again in agreement with Bajardi, that in many of such "transformation cases" in our series the images observed through the colposcope have decided the diagnosis more easily than the cytological findings in smears, and also, that some of these cases were correctly diagnosed only by histology.

Ectopy, ectropion and epidermization are not exceptions to the rule of unexpected pitfalls when studying smears for cancer detection. Irregularity in nuclear size and/or shape, nuclear enlargement, hyperchromasia, etc., including dyskaryotic changes as mentioned by von Haam, are not infrequent (smears reported as Class II or even as Class III). It means to us that we must be alert and watch the patient, although exfoliative cytology in all of our cases became normal (Class I) after inflammation, infection, etc., were treated or a diathermocoagulation was performed.

Luba A. Turnbull, Montreal, Quebec, Canada: It is not so difficult to diagnose an ectropion of the cervix by cytology when one can see an increased number of endocervical cells on the slides, for in the normal condition of the cervix we do not usually see so many of these cells.

But when a question arises concerning cytological diagnosis in the ectopy, and I believe many cytologists agree with me when I say that this condition of the cervix is difficult to differentiate from the ectropion, we proceed to make a clinical examination, sometimes utilizing colposcopy or even biopsy to arrive at a correct diagnosis of ectopy.

The epithelization of the cervix occurs after a prolonged inflammatory disease or a traumatic defect of the cervix. This condition of the cervix, like an ectopy, we cannot recognize with a high degree of accuracy in cervical smears.

Of course in the smears one will observe many different epithelial cells, basal or parabasal, metaplastic or spindle type. A smear may be described as inflammatory, but again without clinical examination we cannot always be fully confident about the diagnosis.

Wolfgang Walz, Heidenheim/Brenz, Germany: The diagnosis of ectopy or ectropion and epidermization cannot be made with certainty by means of exfoliative cytology alone. The qualitative and quantitative signs may be present, but they do not have to be. In extensive epidermization zones and ectopies, we frequently found only an increased number of lymphocytes, leukocytes and mucus in addition to normal superficial and intermediate cells, disregarding the few parabasal and cervical cells. Conversely, in a normal appearing cervix an increasing number of cervical and metaplastic cells may be found. In our opinion, the factor of uncertainty is too great to enable us to make a definite diagnosis by means of cytology alone. We want to emphasize the view of Bajardi, that the combination of cytology with colpomicroscopy helps in arriving at the diagnosis.

It appears to us that only with the aid of cytology may one exclude the presence of a malignant lesion with almost a 100 per cent certainty.

Closing Remarks

Friedrich Bajardi: To Gaudefroy: Certainly the rather high number of exfoliated columnar cells in smears may be explained by partial epidermization of an ectopy or an ectropion. In my own 100 cases the epidermization has, however, already been completed, i.e., the entire ectocervix has been covered again by squamous epithelium. Even colposcopically, no remaining islets of the ectopy or of the ectropion respectively, from which an exfoliation of cylindrical cells could have been possible, could be found. In these complete epidermizations (as already mentioned in my paper), a qualitatively changed epithelium, retention cysts, and openings of cervical glands have been the colposcopically discernible findings.

Ekkehard Kofler, Hans Kremer and Rudolf Ulm: Generally the discussants agree with our findings. Certainly we do try to confirm our cytological diagnosis by the other methods for early cancer detection (colposcopy and colpomicroscopy). We are then in a position to exclude malignant growth with a high degree of diagnostic accuracy.

The Symposia by Correspondence of Acta Cytologica

Introductory Remarks

The Symposia of *Acta Cytologica* are *held entirely by correspondence* and contain international discussions of scientific problems of interest to the exfoliative cytologist.

System for Selecting Subjects for Symposia: From recommendations received, the Editorial Office will draw up the list of subjects and will publish these subjects in *Acta Cytologica,* under the heading: Future Symposia.

The final detailed program will be published in *Acta Cytologica* immediately preceding the one where these topics are to be considered, under the heading: *The Next Symposium.*

Instructions for Authors: Each problem will be introduced by a *Main Speaker* or *Speakers.* These principal papers will then be considered by persons identified as *Discussants.* As a general rule, 200 words each will be allocated for the contributions of the Discussants. The Discussants are requested *to strictly restrict their contributions to the discussion of the main papers.* Discussions which are not directly related to the main paper cannot be accepted. It is suggested that the Discussants prepare their contributions in such a manner that the reader may gain the impression of an actual round table conference. The Main Speakers will then be given the opportunity to make Closing Remarks. The Closing Remarks of the Main Speakers should be limited to the answering of questions raised in the discussion and to other directly related information.

Photomicrographs and tables may be reproduced. The photomicrographs and tables should be submitted in glossy photographic prints, preferably in the size of 3×4 inches and should show a proportional 10μ scale on its reverse side. *Each figure should be accompanied by a comprehensive caption.*

Every cited opinion or publication should have a reference in the bibliography.

Deadline for Contributions: The Editorial Office will set deadlines for each written symposium.

These will include:

1. deadline for agreements to contribute.
2. deadline for main papers.
3. deadline for discussions.
4. deadline for closing remarks.

LES SYMPOSIA PAR CORRESPONDANCE DES ACTA CYTOLOGICA

Les *Symposia par Correspondance* des *Acta Cytologica* présentent des discussions internationales sur des problèmes scientifiques intéressant le cytologiste exfoliative.

Système du choix des sujets pour les symposia: En partant des propositions, et sous la rubrique: Futurs Symposia, le bureau de rédaction dressera la liste des sujets principaux qui seront publiés dans les *Acta Cytologica.*

Le bureau de rédaction établira le programme définitif et détaillé des discussions qui sera publié dans les *Acta Cytologica* précedant immédiatement le symposium, sous la rubrique *Prochain Symposium.*

Recommandations pour les auteurs: Chaque sujet principal sera présenté par un Rapporteur Général ou des Rapporteurs. Ces mémoires principaux seront alors soumis aux Participants à la Discussion. En règle générale 200 mots aux Participants à la Discussion. Les membres et invités prenant part aux discussions sont invités à *limiter strictement leurs interventions aux discussions des sujets principaux.* De discussions qui n'ont pas de rapport direct avec le sujet principal *ne pourront être acceptées.* Il est recommandé que les discussions soient rédigées d'une manière telle que le lecteure aît l'impression d'assister à une discution réelle de table ronde. Les Rapporteurs Généraux pourront clôturer les discussions par un nombre illimité de remarques. Les Remarques de Clôture du Rapporteur Général devront se limiter à la réponse aux questions soulevées dans les discussions et aux autres informations éventuelles ayant un rapport direct avec le sujet.

Des microphotos et graphiques pourront être reproduits. Les microphotos et les graphiques doivent être présentés sur du papier brillant, de préference dans le format 7.5×10 cm. *Chaque figure devra être accompagnée d'une legende explicative précise.*

Chaque opinion ou publication citée dans le texte doit avoir sa réference dans la bibliographie.

Dates limite pour les collaborations: Le bureau de rédaction, fixera des dates limite comprenant:

1. un délai pour l'acceptation des collaborations,
2. un délai pour les sujets principaux,
3. un délai pour les discussions,
4. un délai pour les remarques de clôture.

The Symposia by Correspondence of Acta Cytologica

DIE SCHRIFTLICHEN SYMPOSIEN DER ACTA CYTOLOGICA

Die schriftlichen Symposien der *Acta Cytologica* befassen sich auf internationaler Basis mit wissenschaftlichen Problemen, die für den Exfoliativ-Zytologen von Interesse sind.

System der Thema-Auswahl für die Symposien: Die Schriftleitung stellt auf Grund von Thema-Vorschlägen eine Liste von Haupt-Themen zusammen, und gibt diese Liste unter dem Titel: Zukünftige Symposien bekannt.

Die Schriftleitung bereitet das Programm mit allen Einzelpunkten vor, und veröffentlicht dieses Programm in dem Heft, das dem betreffenden Symposion vorausgeht, unter dem Titel: *Das Nächste Symposion.*

Instruktionen fur Autoren: Jedes Thema wird von einem oder mehreren Referenten behandelt. Diese Referate werden dann von Diskussions-Vortragenden besprochen. Im allgemeinen werden Diskussions-Vorträge auf 200 Worte. Die Diskussionsvortragenden sind gebeten, sich in ihren Beiträgen *streng an das Hauptreferat zu halten.* Diskussionbeiträge, die sich nicht an das Hauptthema halten, *können nicht berücksichtigt werden.* Es wird vorgeschlagen, dass die Diskussionsvorträge in einem Stil abgefasst sind, dass der Leser den Eindruck gewinnt, als ob es sich um eine Diskussion am runden Tisch gehandelt hätte. Die Referenten erhalten dann die Gelegenheit, Schlussbemerkungen zu machen. Die Schlussbemerkungen der Referenten sollen sich nach Möglichkeit auf die Beantwortung von Diskussionsfragen beschränken.

Mikrophotographien und Tabellen können abgedruckt werden. Die Photographien sind auf Hochglanzpapier, und möglichst in der Grösse 7.5 × 10 cm erbeten und soll ein proportionales 10μ Zeichen auf der Rückseite haben. *Jede Abbildung muss von einem erklärenden Untertitel begleitet sein.*

Jede zitierte Ansicht oder Publikation muss eine Referenz in der Bibliographie haben.

Termine für Beiträge: Die Schriftleitung setzt Termine für die Schriftlichen Symposien fest. Die folgenden Termine werden bekanntgegeben:

1. Termin für Erhalt der Beitrags-Zusagen,
2. Termin für Erhalt der Hauptreferate,
3. Termin für Erhalt der Diskussions-beiträge,
4. Termin für Erhalt der Schlussbemerkungen.

SIMPOSIUM ESCRITO DE ACTA CYTOLOGICA

El simposium escrito de *Acta Cytologica* contiene discusiones internacionales sobre problemas científicos que son de interés para el citólogo exfoliativo.

Sistema de selección de materias para el simposium: Con sugestiones recibidas, la oficina editorial confeccionará una lista de los temas más interesantes, lista que será publicada en *Acta Cytologica* con dos numeros de antícipación a la fecha de su posible publicación, bajo el epígrafe de "Simposium Futuros."

La Oficina Editorial confeccionará y publicará una lista detallada del programa de la discusión en el número de *Acta Cytologica* immediatamente anterior a aquel en que han de ser incluidos los temas, bajo el epígrafe de: El Proximo Simposium.

Instrucciones a los Autores: Cada problema deberá ser presentado por un ponente o ponentes. Estos trabajos principales serán entonces discutidos por los comunicantes. Como regla general, se permite un máximo de 200 palabras para las contribuciones de los comunicantes. Se suplica a los comunicantes *ajustar estrictamente sus comunicaciones a la discusión de los trabajos principales.* Las discusiones que no estén directamente relacionadas con el trabajo principal *no podrán ser aceptadas.* Se sugiere que los comunicantes realicen sus contribuciones de manera tal que el lector tenga la impresión de estar ante una verdadera mesa redonda. Al ponente principal se le da la oportunidad de hacer rectificaciones finales ilimitadas. Las rectificaciones finales de los ponentes deberán limitarse a contestar las preguntas aparecidas a lo largo de la discusión asi como a otras directamente relacionadas con el tema.

Pueden reproducirse microfotografías y tablas: una página por cada trabajo principal y un máximo de media página por discusion. Las microfotografías y tablas deberán enviarse en forma de copias fotografícas amplias. A ser posible de 3 × 4 pulgadas (7.5 × 10 cms.) *Cada figura deberá acompañarse de su correspondiente leyenda.*

Toda opinión o publicación citada deberá tener su correspondiente referencia en la bibliografía.

Fechas para los contribuciones: La Oficina Editorial, fijará fechas límite absolutas para cada simposium escrito. Estas incluirán:

1°. Fecha límite para acuerdo de contribución,
2°. Fecha límite para las ponencias,
3°. Fecha límite para las discusiones,
4°. Fecha límite para las anotaciones finales.